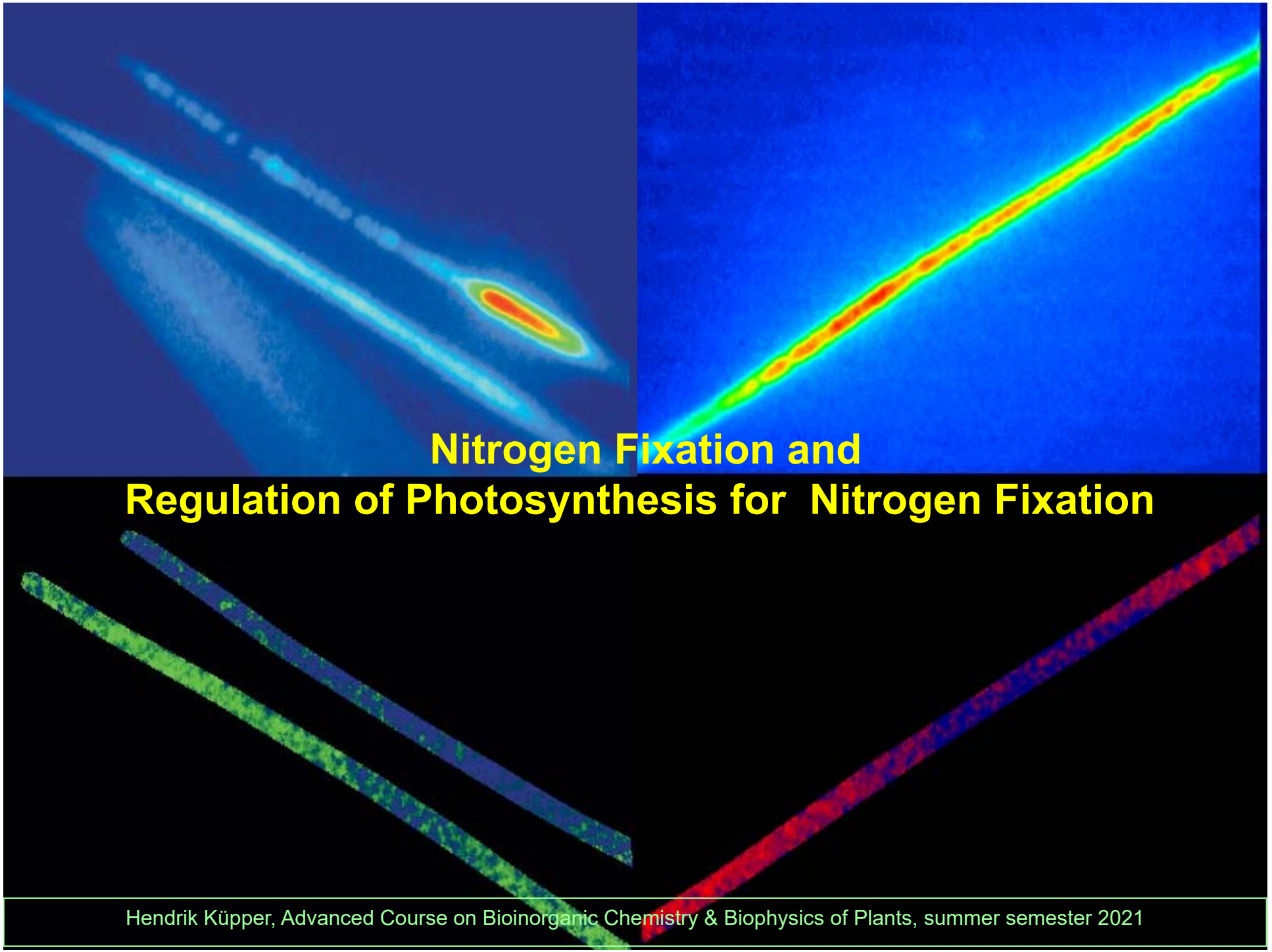
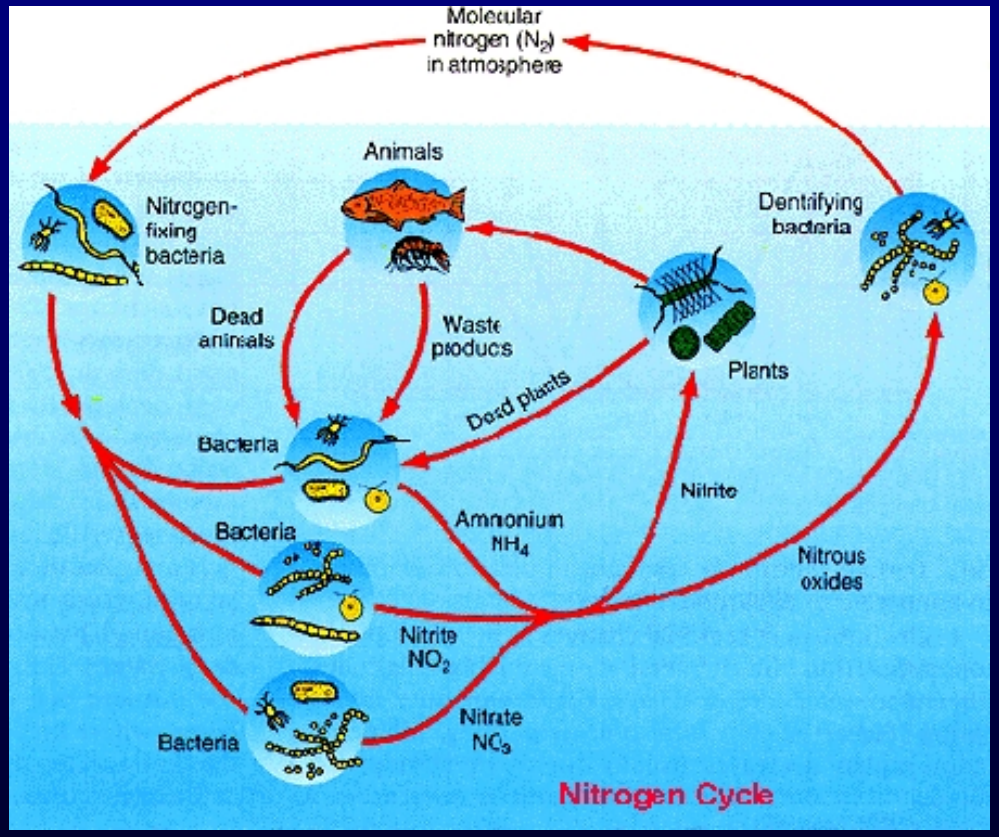


Nitrogen Fixation and Regulation of Photosynthesis for Nitrogen Fixation

Part I:
Nitrogen fixation

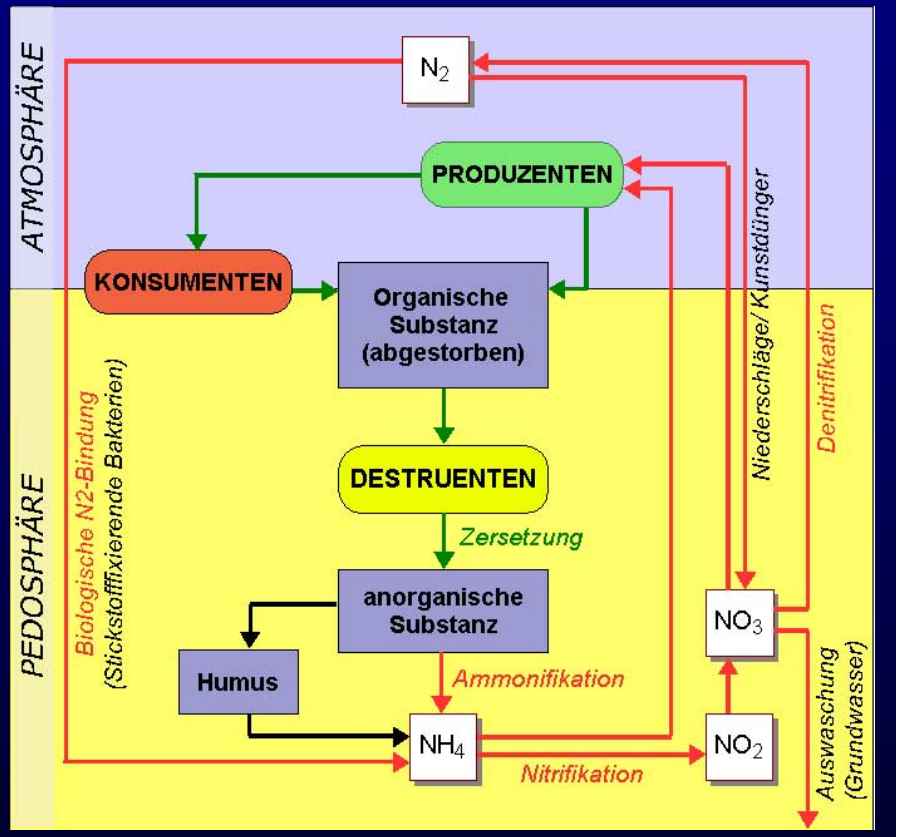
Nitrogen cycle (I): biological processes

Im Wasser



von: www.oceanography.geol.ucsb.edu

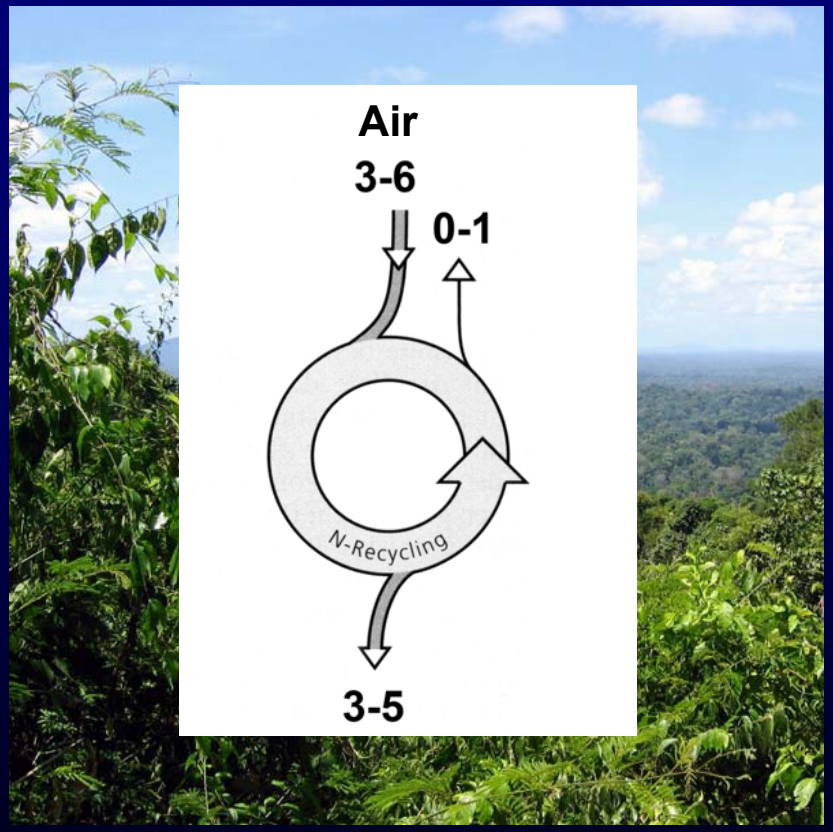
An Land



von: www.hypersoil.uni-muenster.de

Nitrogen cycle (II): natural vs. anthropogenic processes

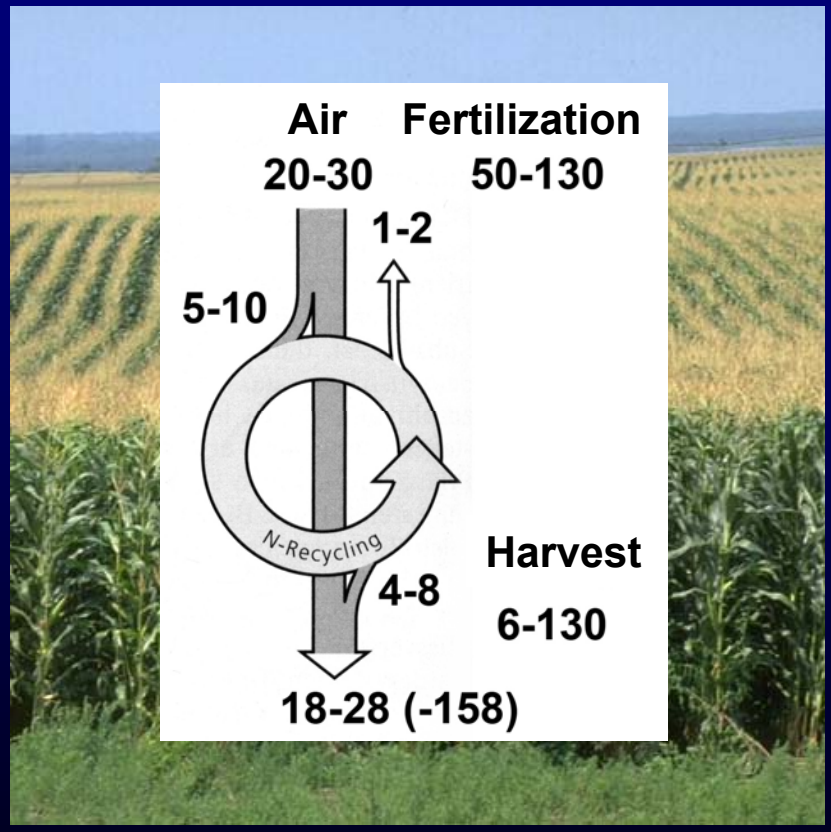
Lightnings, biol. N_2-fixation



NO_3^-, Herbivores, Erosion

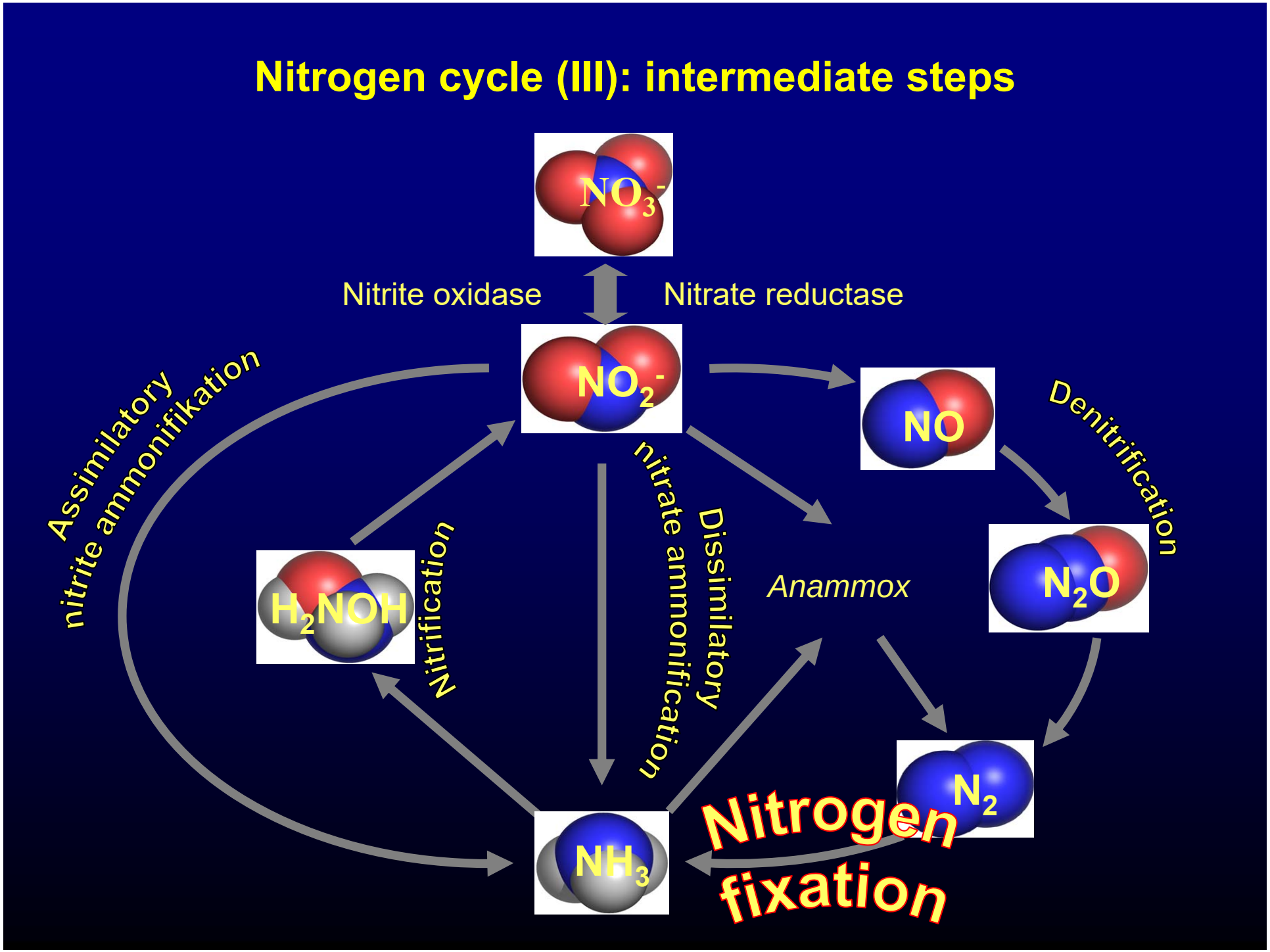
kg N / (ha * Jahr)

NO_x, organ. / mineral. Fertilizers

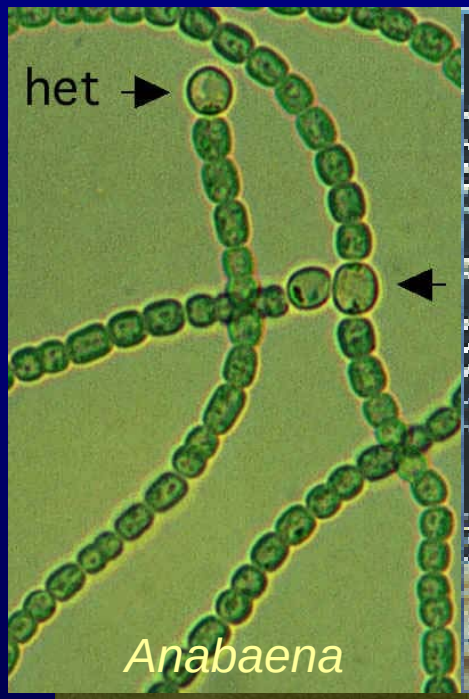


NO_3^-, Harvest, Erosion

Nitrogen cycle (III): intermediate steps

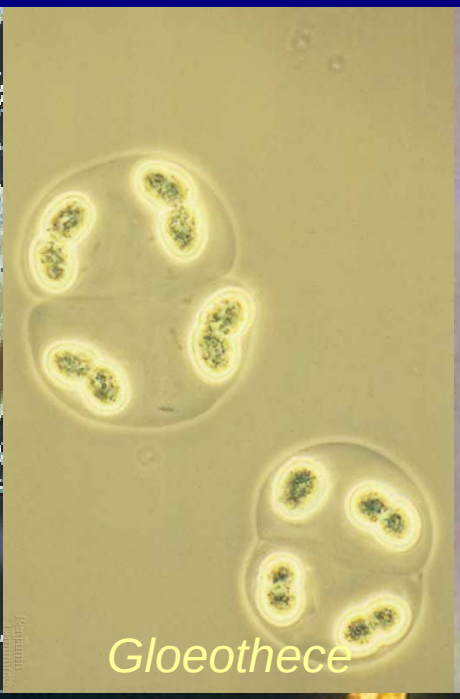


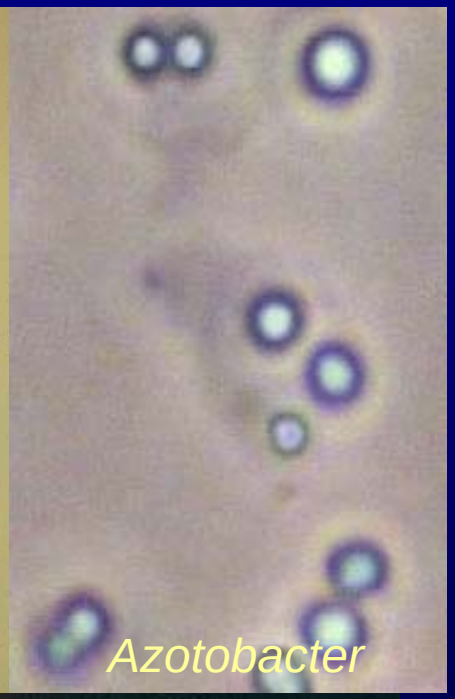
Organisms involved in nitrogen fixation



Anabaena


Trichodesmium


Gloeothece


Azotobacter


Azolla

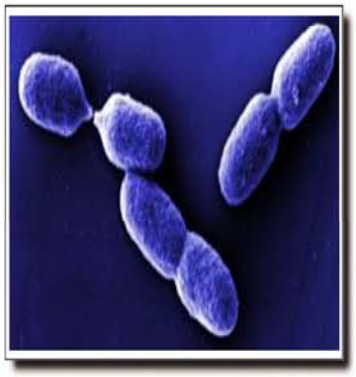

Rhizobien

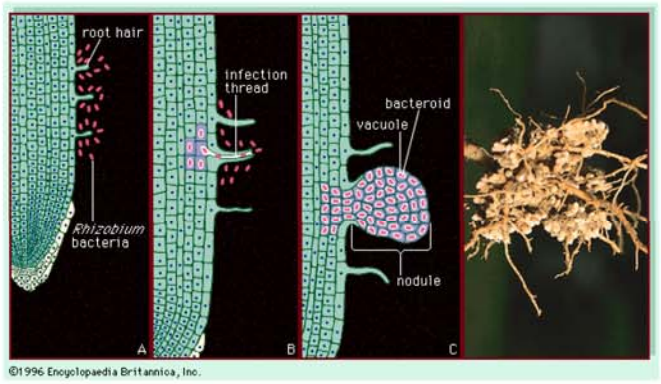
Biological N_2 Fixation

Microorganisms can do the job under „normal conditions“ (T, P)

- free living soil bacteria, e.g. *Azotobacter vinelandii*
- Cyanobacteria with specialized cells, e.g. *Anabaena sp., Nostoc sp.*)
- *Rhizobia* which live in special plant organelles (root noudles)

The process, however, is costly. Plants have to deliver up to 25% of their photosynthetically produced ATP to N_2 fixing bacteria in the root nodules.





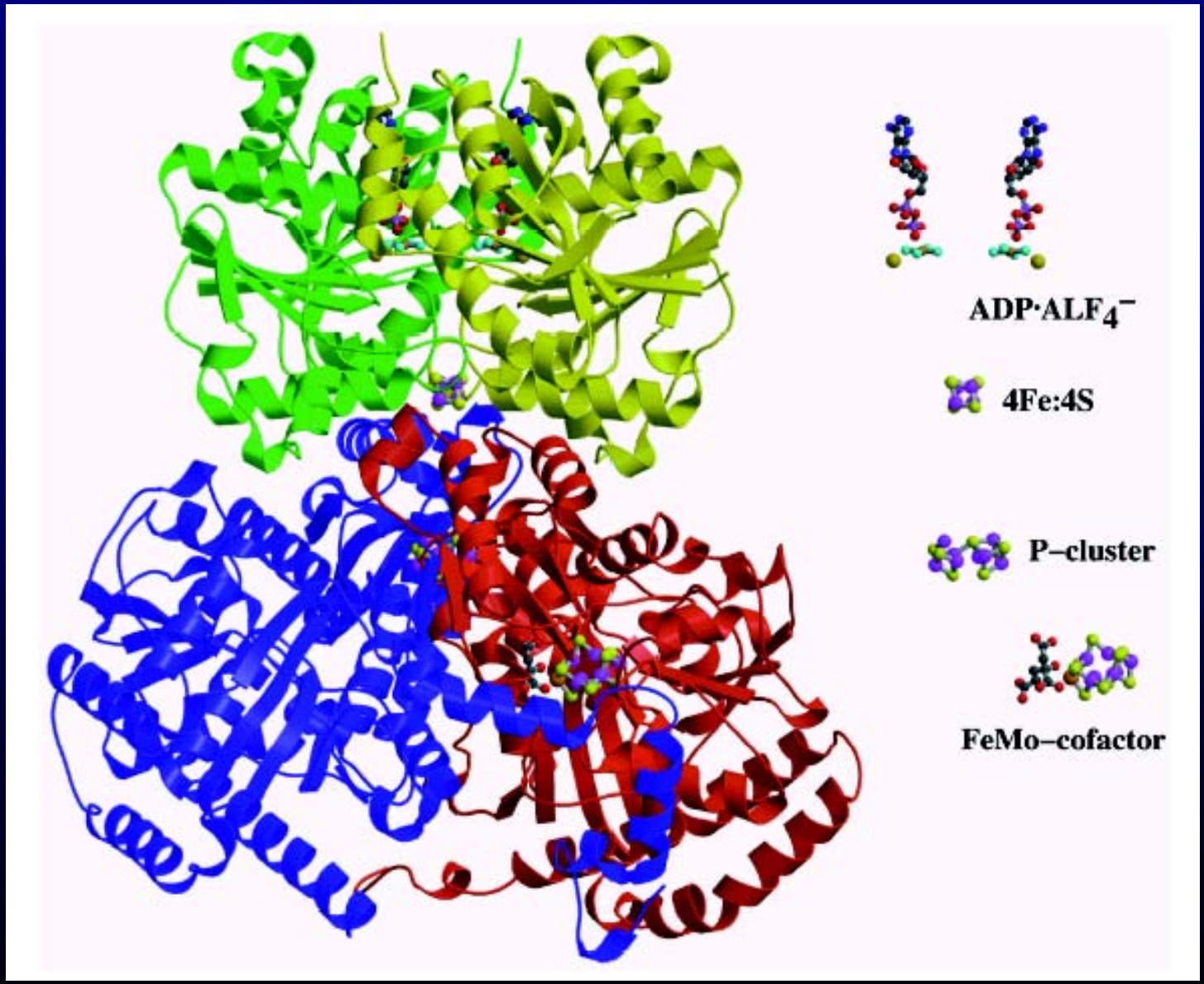
Note: N_2 Fixation is an anaerobic process

Total equation of biological nitrogen fixation

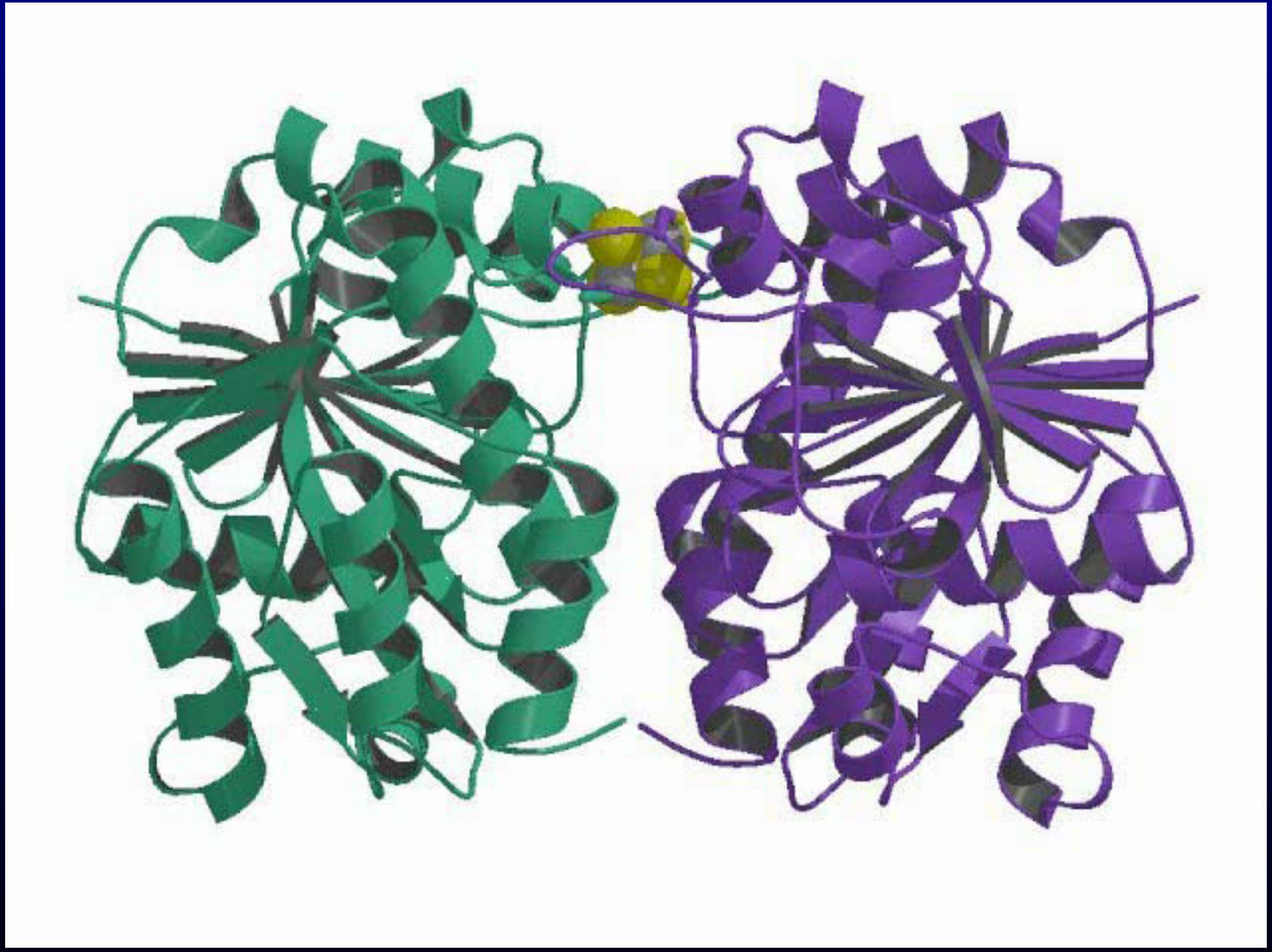
$$N_2 + 8H^+ + 16\ MgATP + 8e^-$$

$$2NH_3 + H_2 + 16\ MgADP + 16\ P_i$$

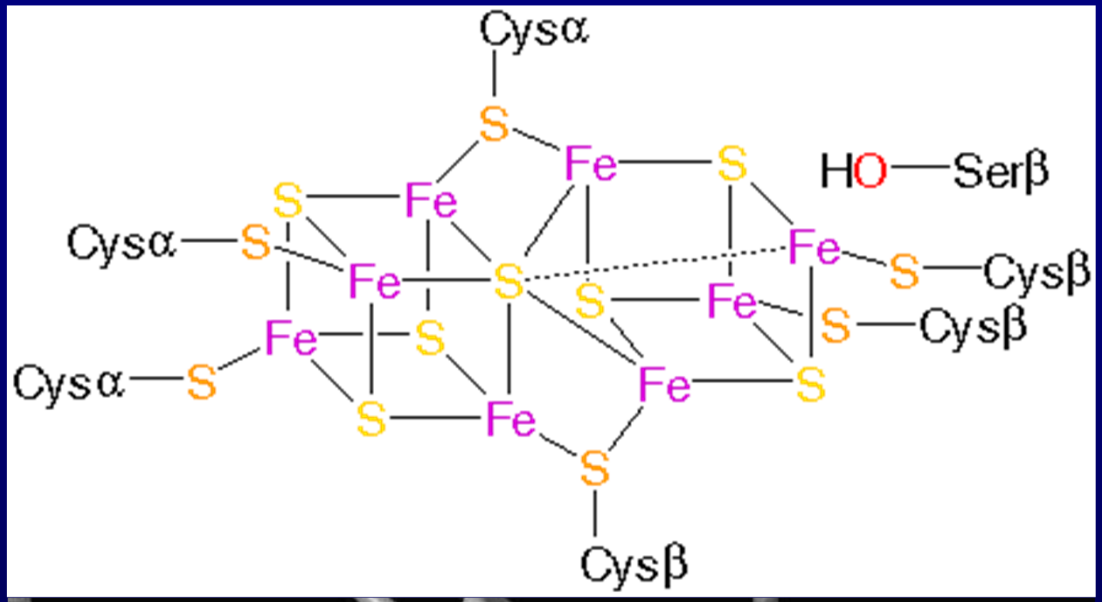
Mechanism of biological nitrogen fixation: nitrogenase of cyanobacteria

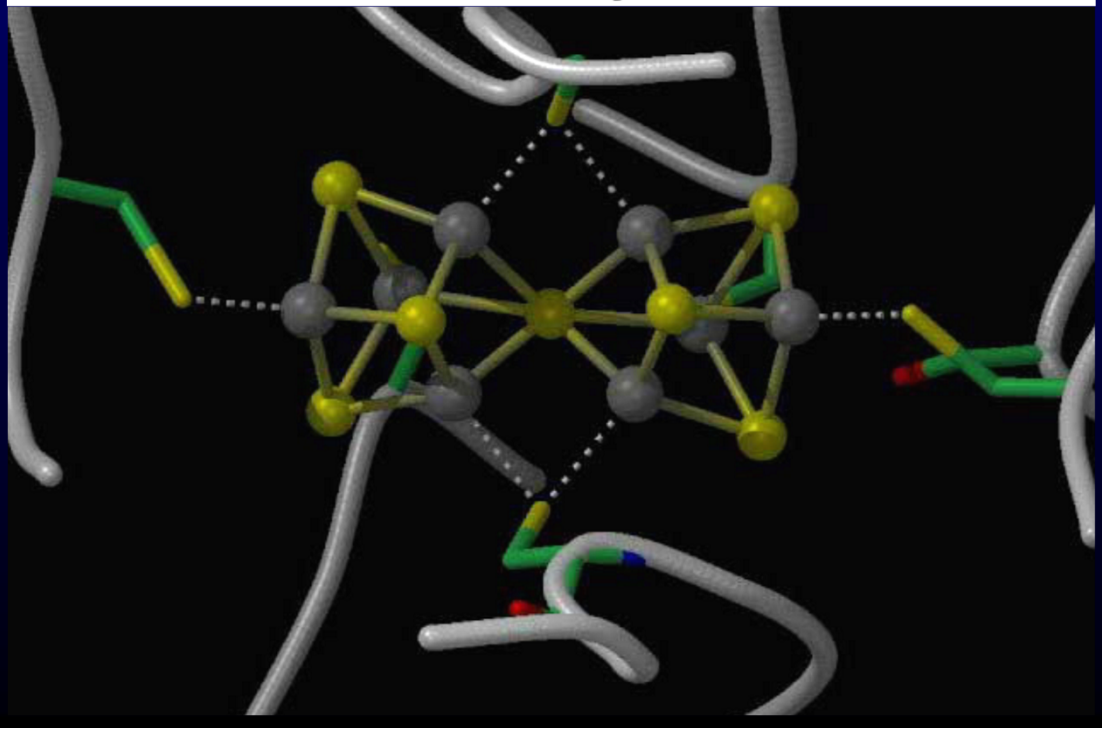


The iron protein

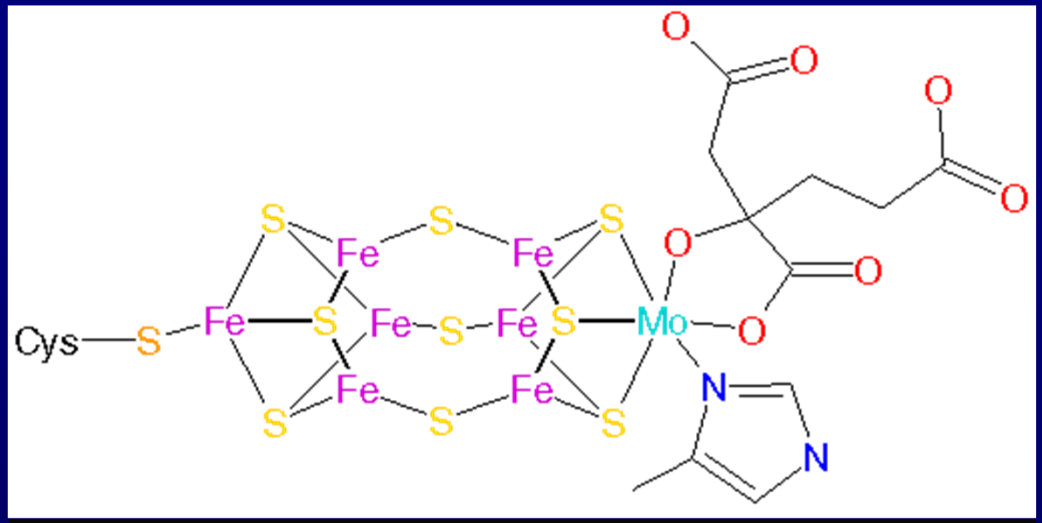


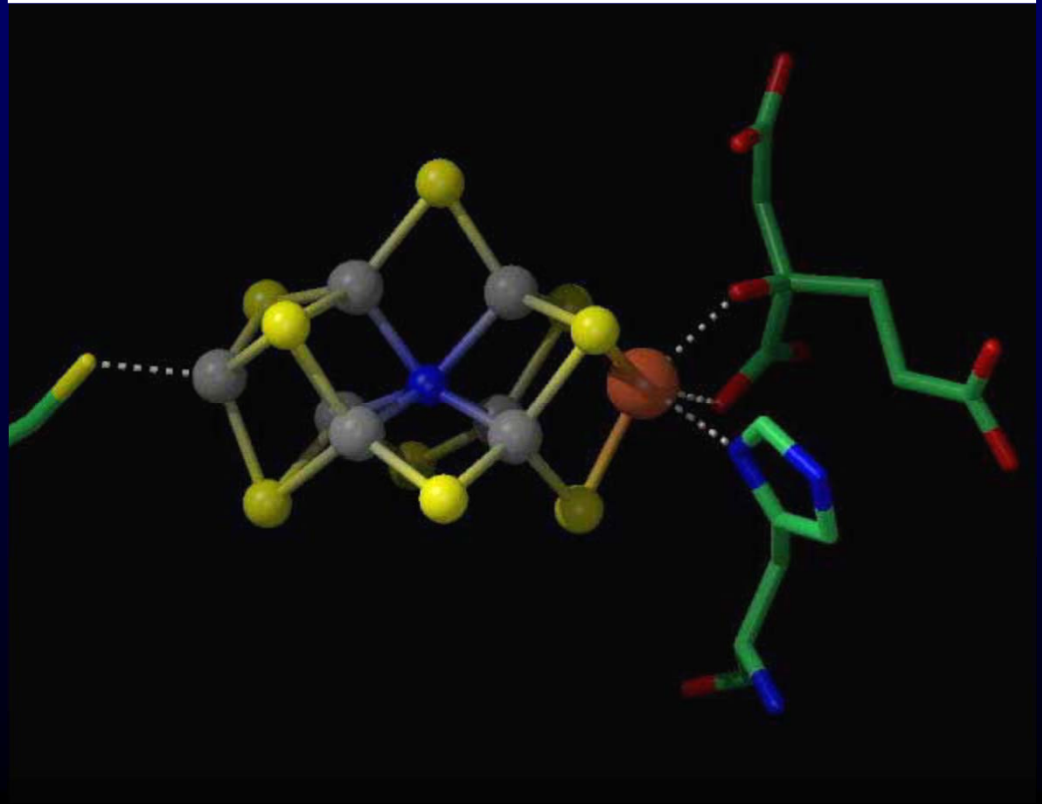
Nitrogenase: P-Cluster





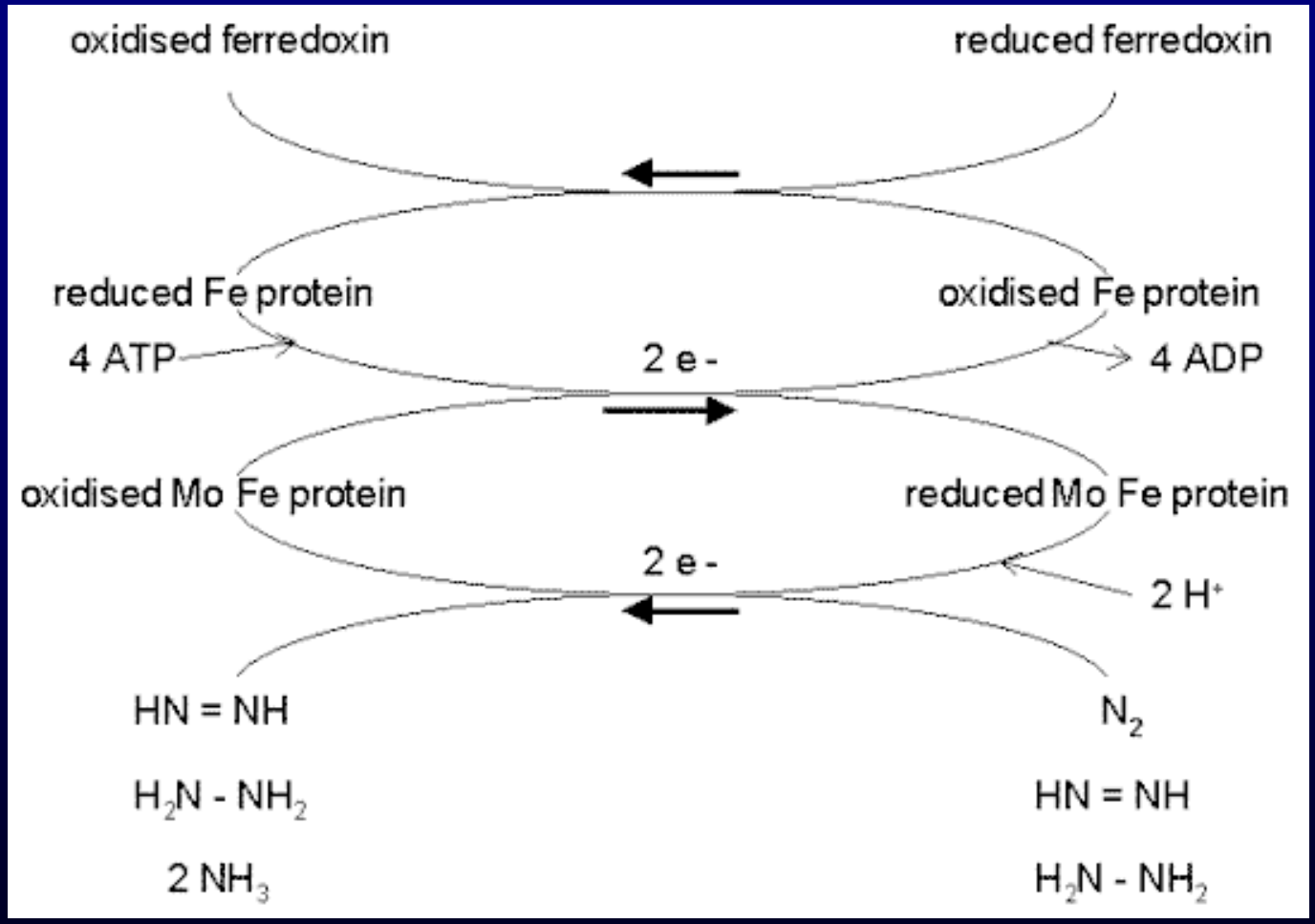
Nitrogenase: M-Cluster



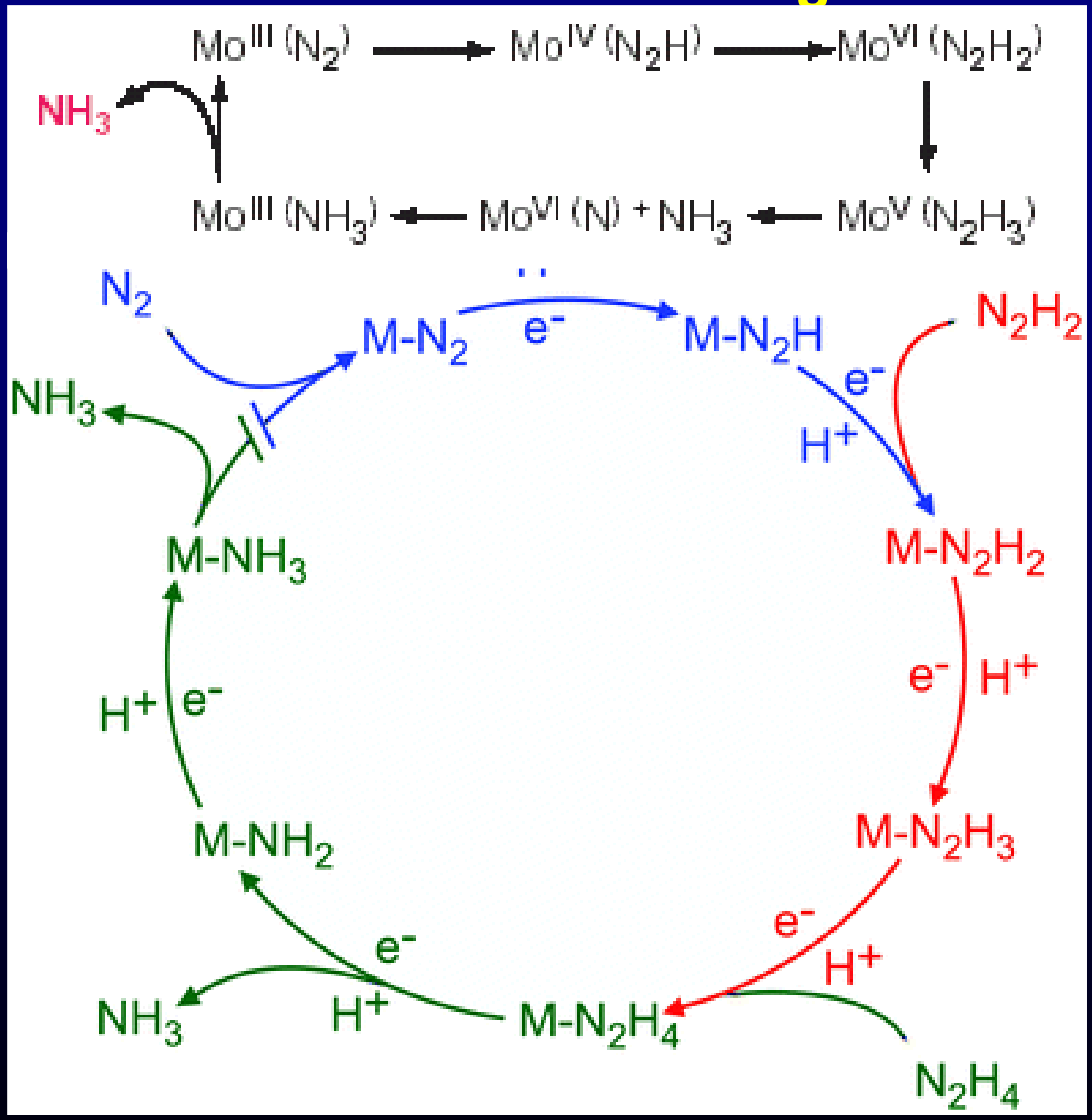


Structure of Mayer and Smith, 1999, 1.6 Å resolution: discovery of the "central nitrogen", meanwhile re-identified as central carbon (Spatzal Thomas; Aksoyoglu Muege; Zhang Limei; et al. 2011, Science 334; Lancaster Kyle M.; Roemelt Michael; Ettenhuber Patrick; et al., 2011, Science 334)

Nitrogenase: Mechanism of nitrogen reduction

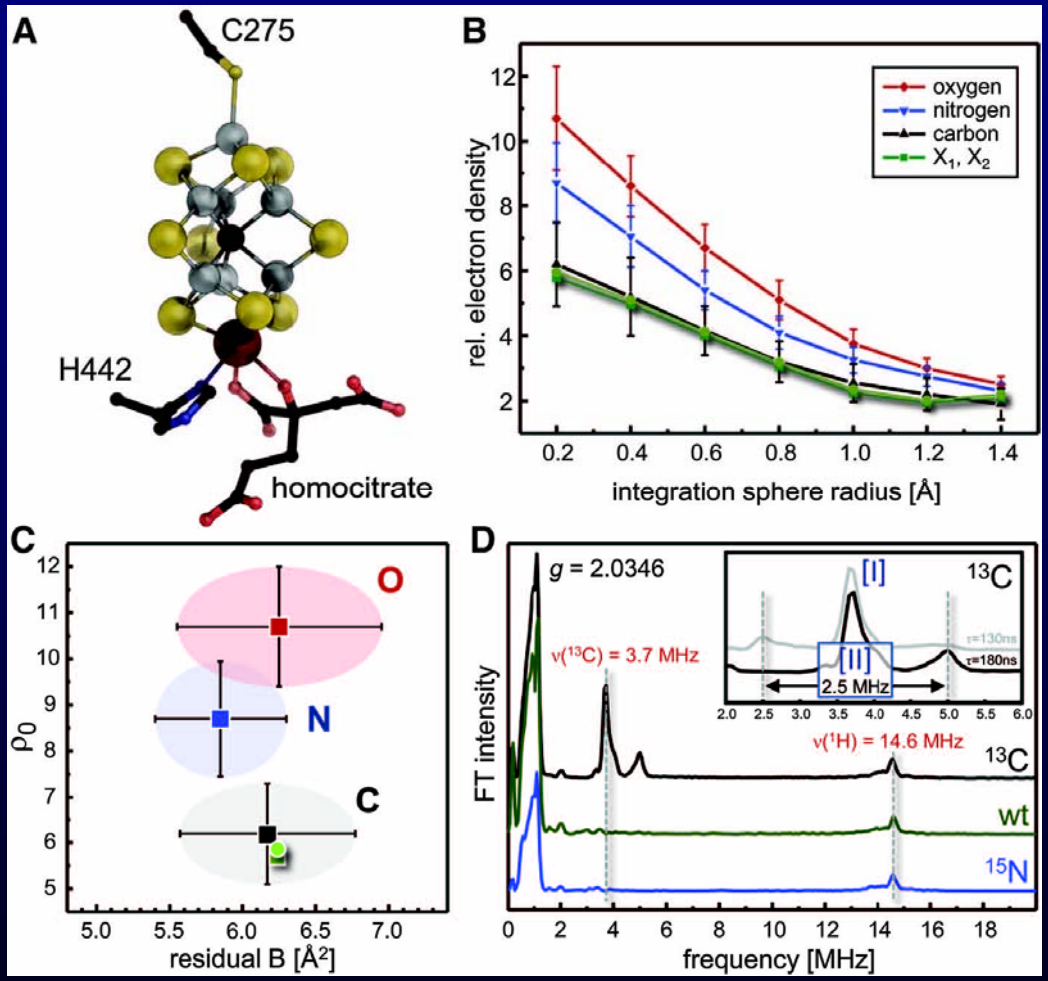


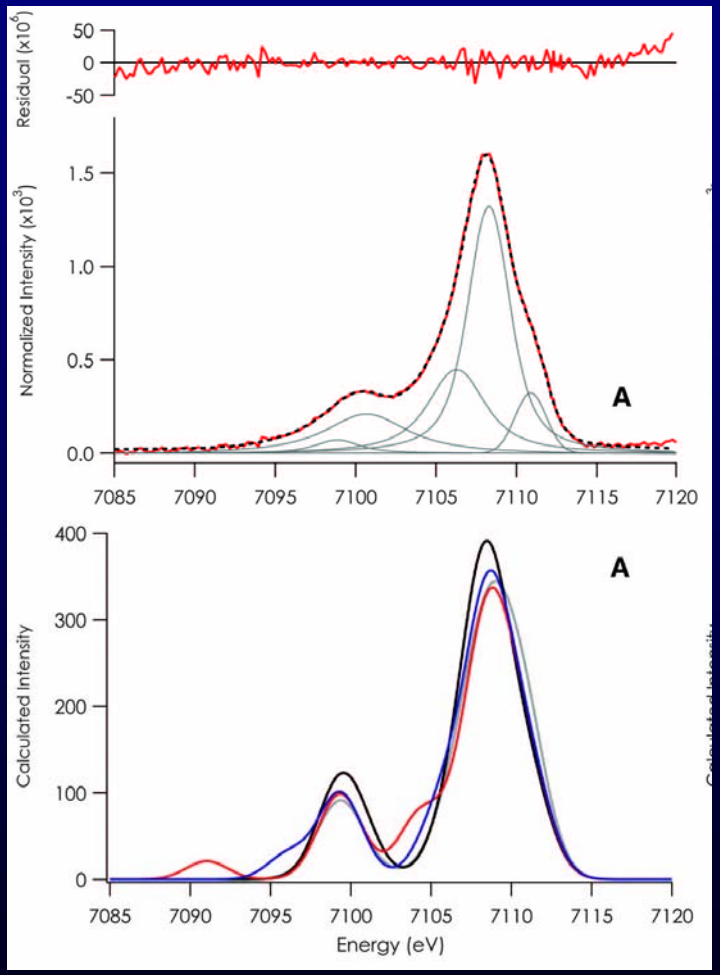
Nitrogenase:
putative intermediates of nitrogen reduction



Barney B_et al (2006) Breaking the N_2 triple bond- insights into the nitrogenase mechanism. DaltonTransactions 2277-84

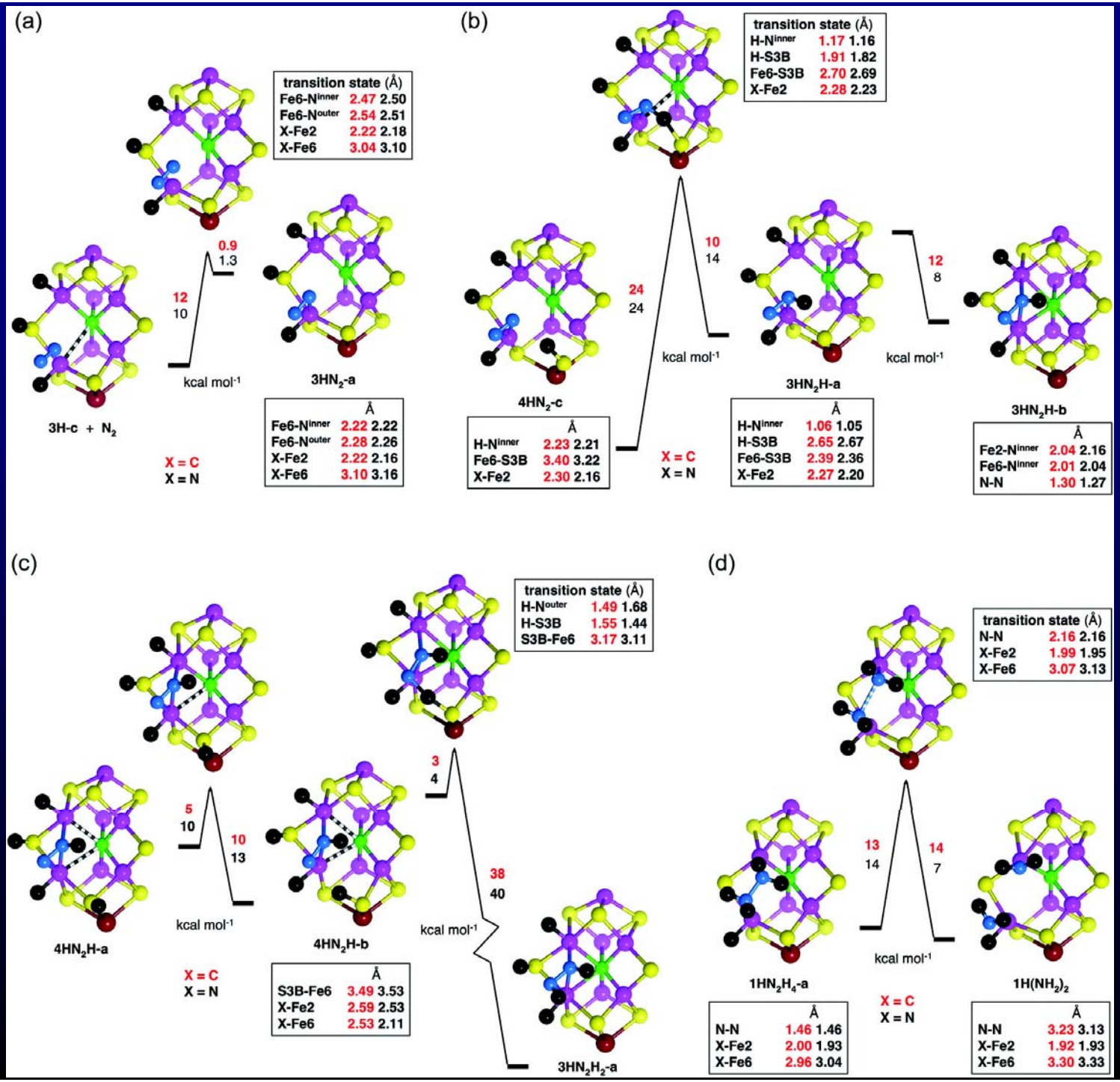
Nitrogenase: central C





Spatzal T et al et Einsle O (2011) Science334, 940-

Lancaster KM_et al et Neese F et DeBeer S (2011) Science334_974-

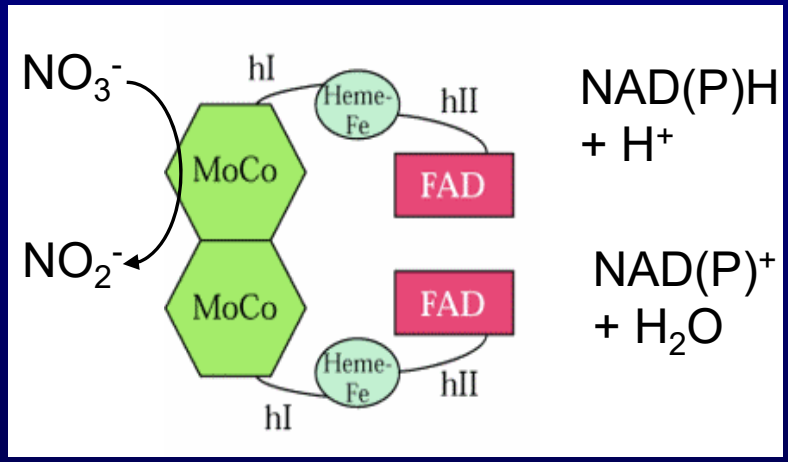

(a)
transition state (Å)
Fe6-N^inner 2.47 2.50
Fe6-N^outer 2.54 2.51
X-Fe2 2.22 2.18
X-Fe6 3.04 3.10
0.9
1.3
12
10
kcal mol-1
3H-c + N2
3HN2-a
Å
Fe6-N^inner 2.22 2.22
Fe6-N^outer 2.28 2.26
X-Fe2 2.22 2.16
X-Fe6 3.10 3.16
X = C
X = N
(b)
transition state (Å)
H-N^inner 1.17 1.16
H-S3B 1.91 1.82
Fe6-S3B 2.70 2.69
X-Fe2 2.28 2.23
10
14
24
24
kcal mol-1
4HN2-c
Å
H-N^inner 2.23 2.21
Fe6-S3B 3.40 3.22
X-Fe2 2.30 2.16
X = C
X = N
3HN2H-a
Å
H-N^inner 1.06 1.05
H-S3B 2.65 2.67
Fe6-S3B 2.39 2.36
X-Fe2 2.27 2.20
12
8
kcal mol-1
3HN2H-b
Å
Fe2-N^inner 2.04 2.16
Fe6-N^inner 2.01 2.04
N-N 1.30 1.27
(c)
transition state (Å)
H-N^outer 1.49 1.68
H-S3B 1.55 1.44
S3B-Fe6 3.17 3.11
5
10
10
13
kcal mol-1
4HN2H-a
4HN2H-b
Å
S3B-Fe6 3.49 3.53
X-Fe2 2.59 2.53
X-Fe6 2.53 2.11
X = C
X = N
3
4
38
40
kcal mol-1
3HN2H2-a
(d)
transition state (Å)
N-N 2.16 2.16
X-Fe2 1.99 1.95
X-Fe6 3.07 3.13
13
14
14
7
kcal mol-1
1HN2H4-a
Å
N-N 1.46 1.46
X-Fe2 2.00 1.93
X-Fe6 2.96 3.04
X = C
X = N
1H(NH2)2
Å
N-N 3.23 3.13
X-Fe2 1.92 1.93
X-Fe6 3.30 3.33

Nitrate reduction

Nitrate reductase in the cytoplasm

$N^{(+V)} \longrightarrow N^{(+III)}$

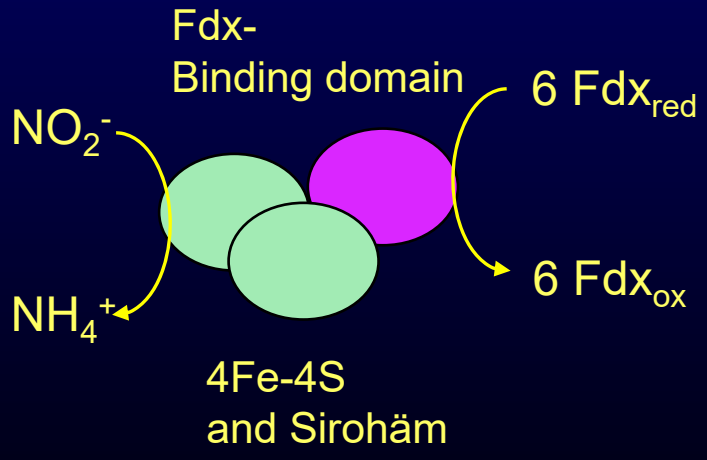
Mo as essential cofactor



Nitrite reductase in the chloroplast stroma

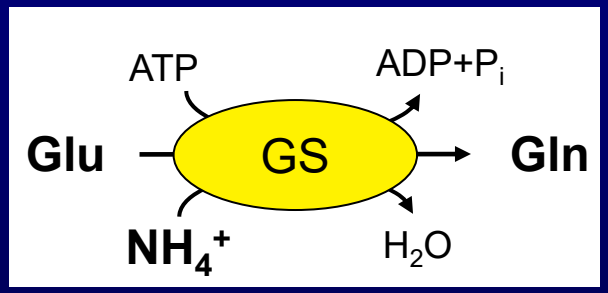
$N^{(+III)} \longrightarrow N^{(-III)}$

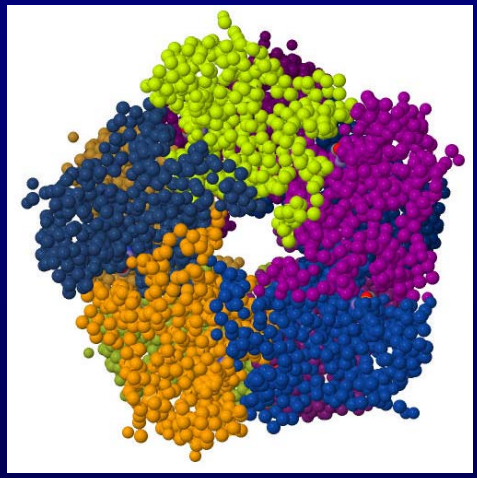
Receives energy directly from the linear electron transport of photosynthesis

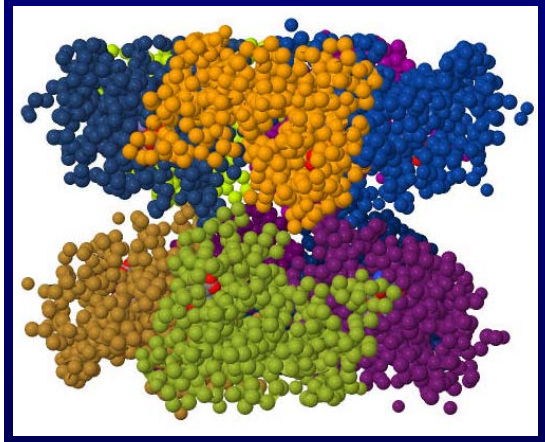


Ammonium assimilation

Glutamine-Synthetase



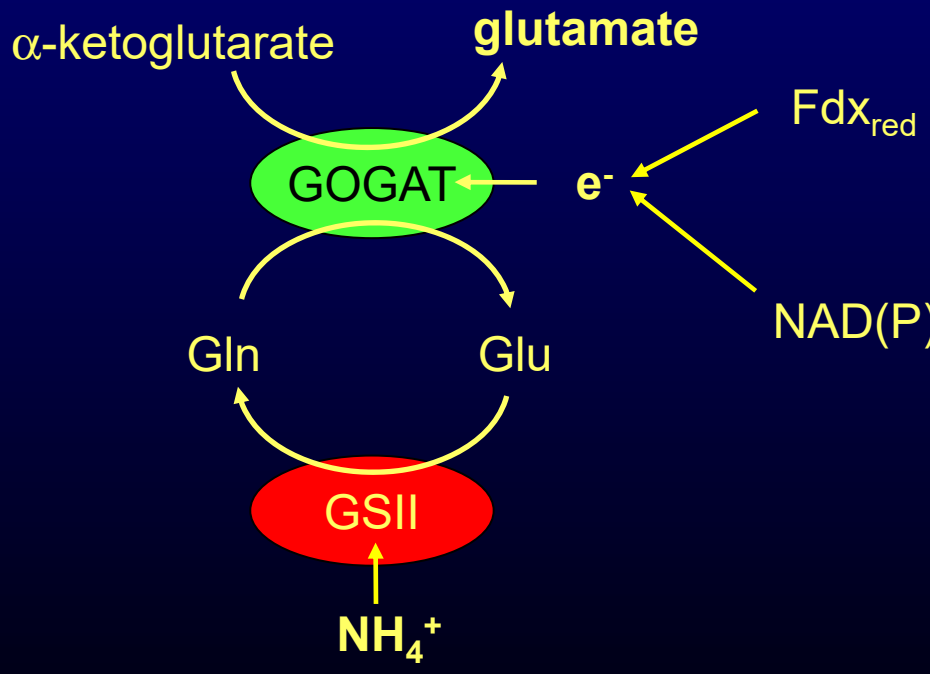




- Decamer with 10 reaction centres at the contact points of the subunits
- Mn as essental cofactor
- GSII in the chloroplast stroma → primary assimilation
- GSI in the cytoplasm → recycling
- Inhibition by phosphinothrizin (Glufosinate, Basta®)

Glutamate synthase = GOGAT

- **G**lutamine **o**xo**g**lutarate **a**mino**t**ransferase
- in plastids
- main pathway of N-Assimilation
- active site: 3Fe4S-cluster combined with flavin mononucleotide (FMN)



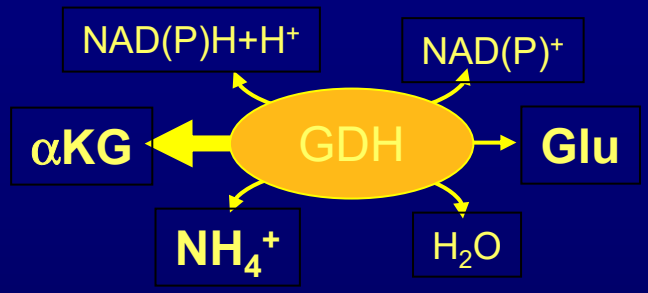
in green **leaves:**

- Primary assimilation
- Photorespiration

in **roots** and cotyledons:

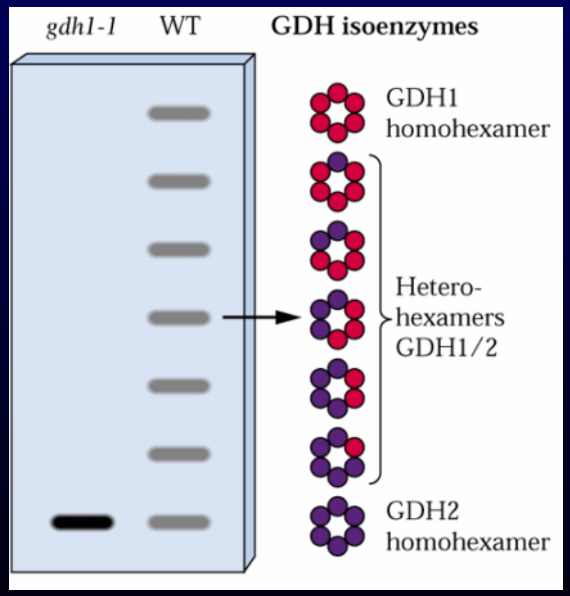
- primary assimilation,
- amino acid recycling

Glutamate dehydrogenase

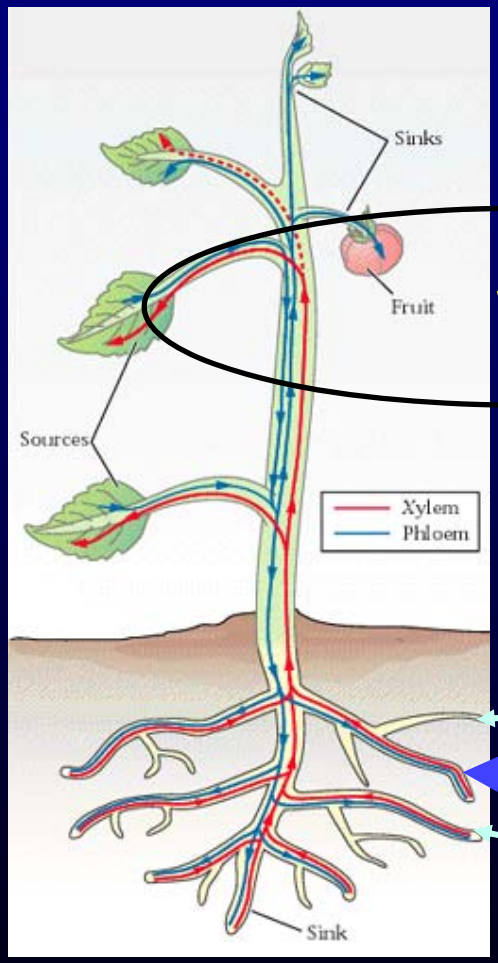


- low affinity for NH_4^+
- predominantly degradation of glutamate
- Cu (and Co ?) as cofactors

- in mitochondria
- 3 isoforms
- GDH1 and GDH2 form homo- or hetero- hexamers



Summary: Nitrogen assimilation in plants



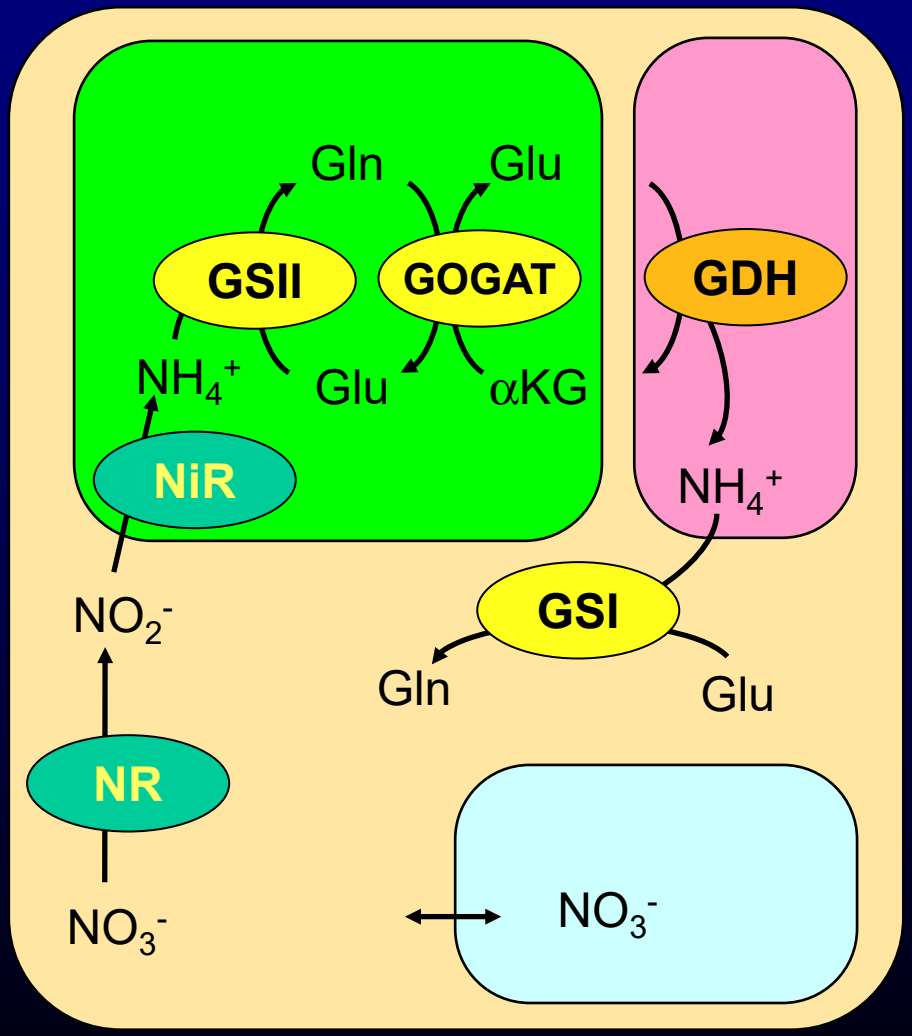
R-NH

NO_3^-

NO_3^-

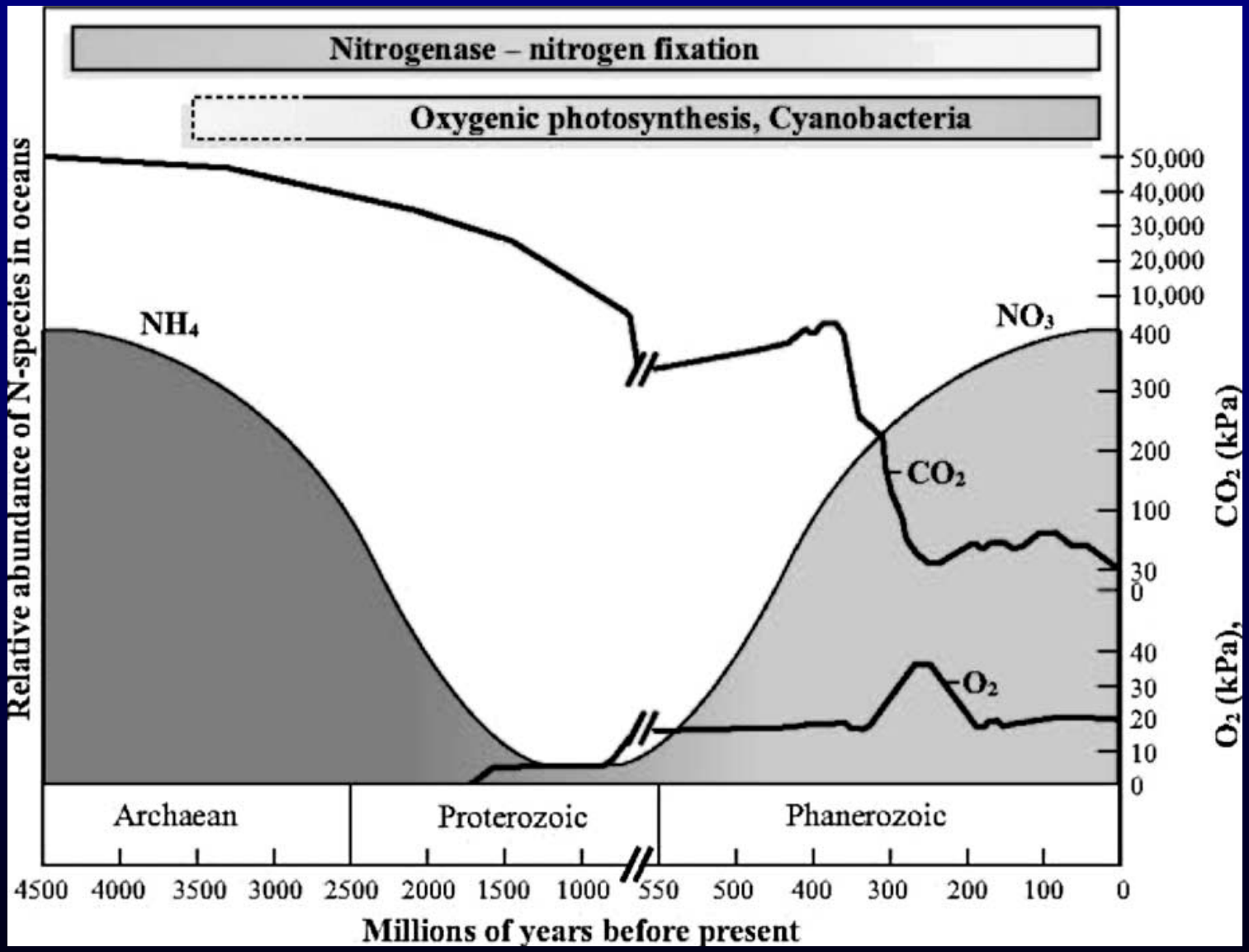
H_2O

NH_4^+



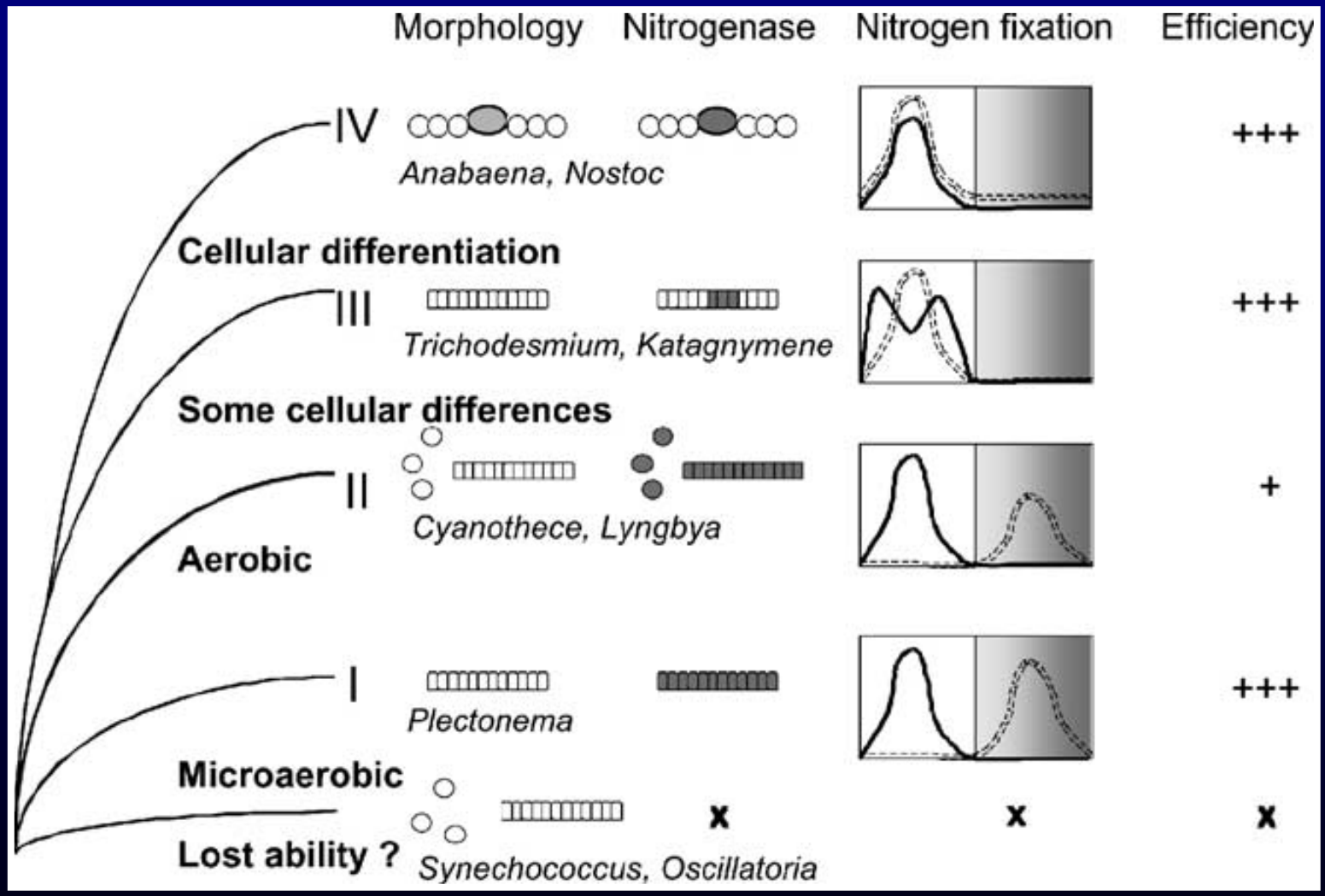
Part II:
Regulation of photosynthesis for nitrogen fixation

Evolution of biological nitrogen fixation in comparison to photosynthesis



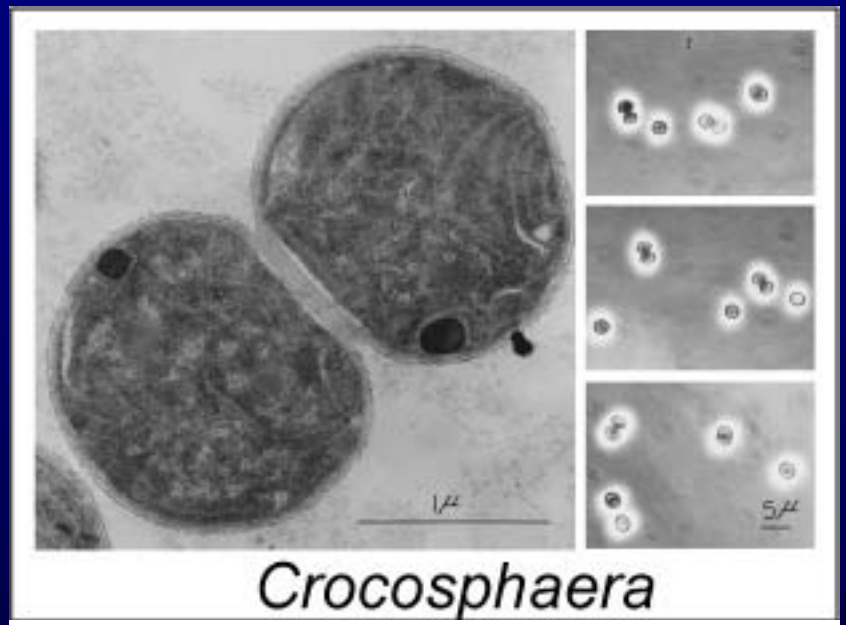
Berman-Frank I_Lundgren P_Falkowski P_2003_Research in Microbiology154_157-164

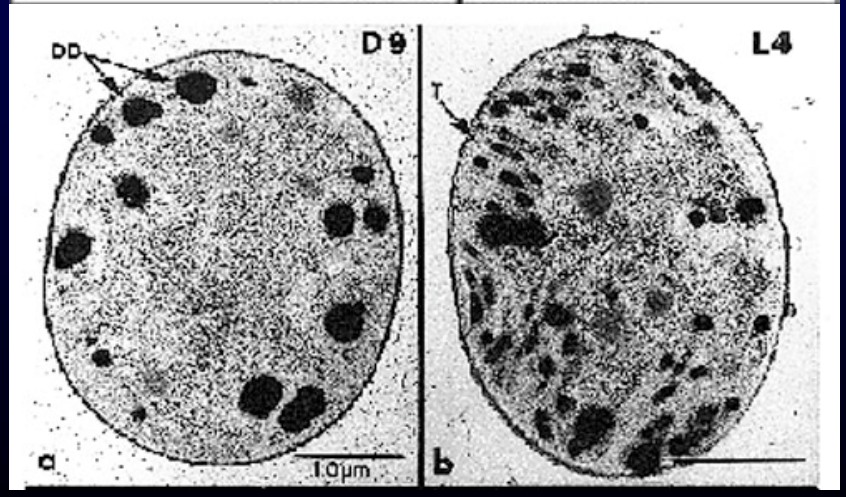
Strategies of photosynthesis regulation for nitrogen fixation in cyanobacteria

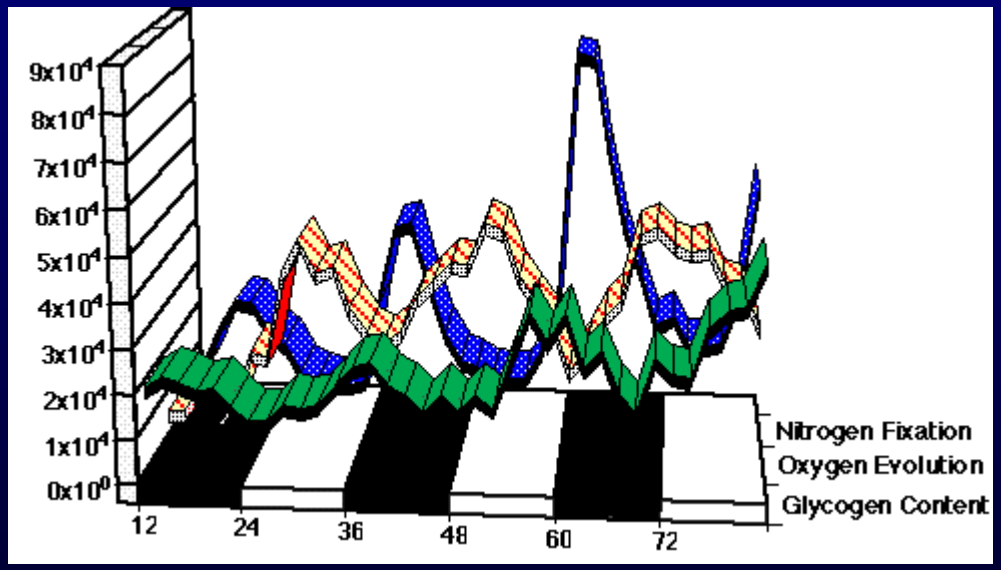


Berman-Frank I_Lundgren P_Falkowski P_2003_Research in Microbiology154_157-164

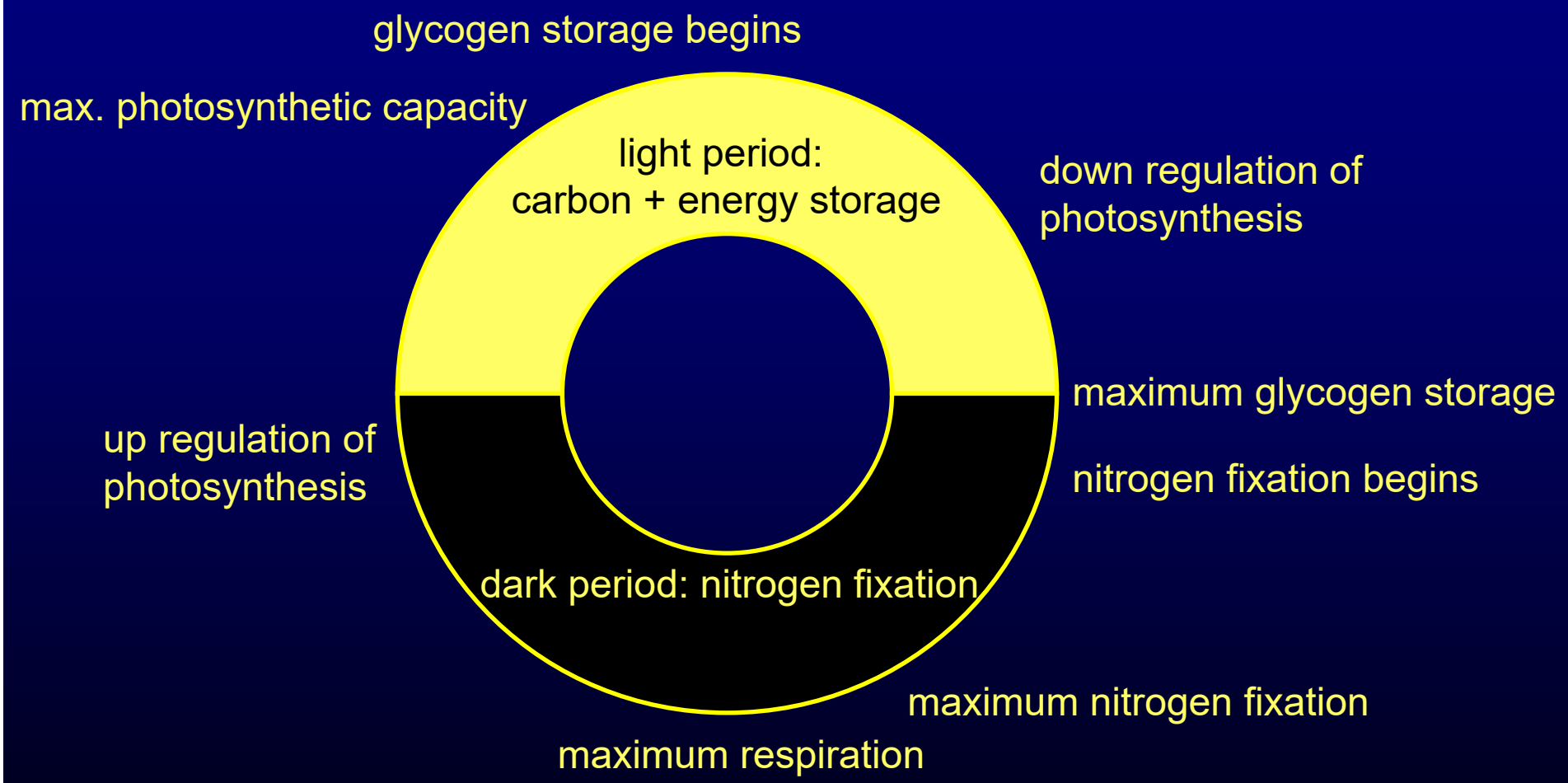
Unicellular cyanobacteria



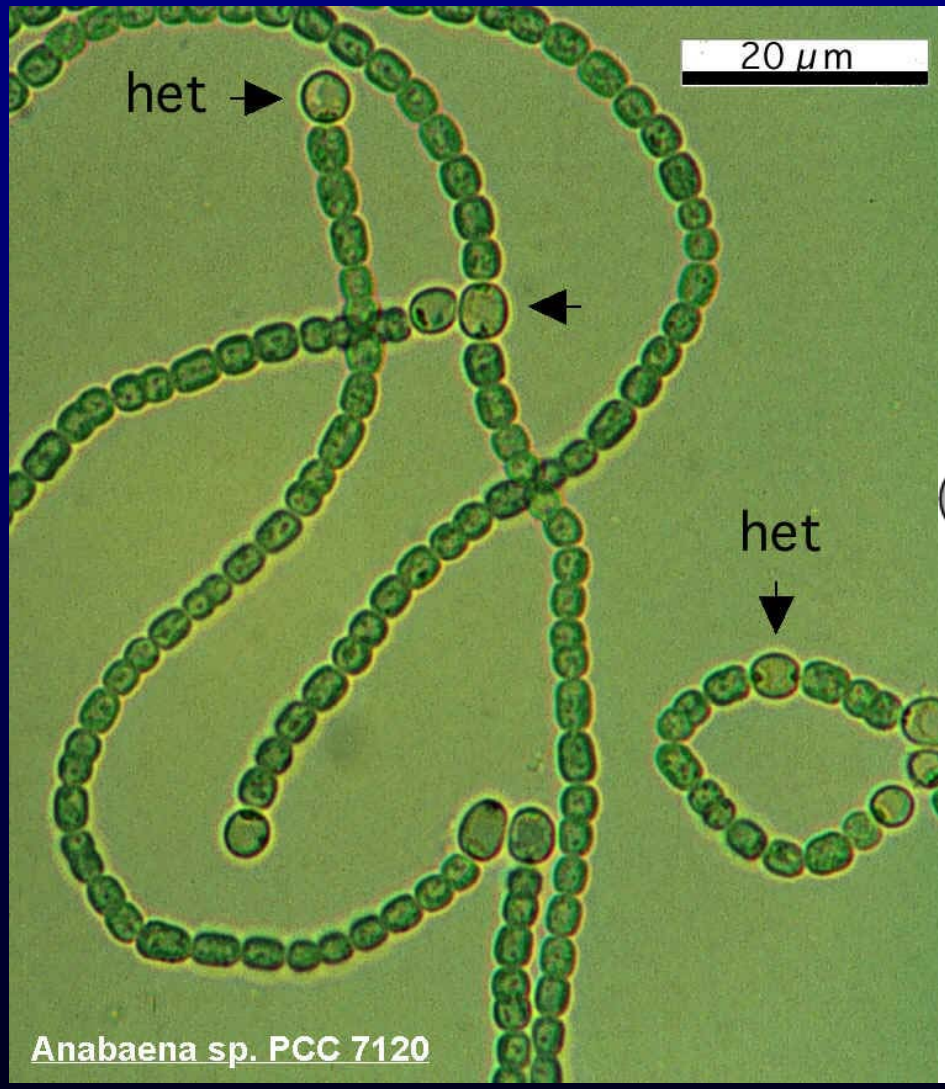




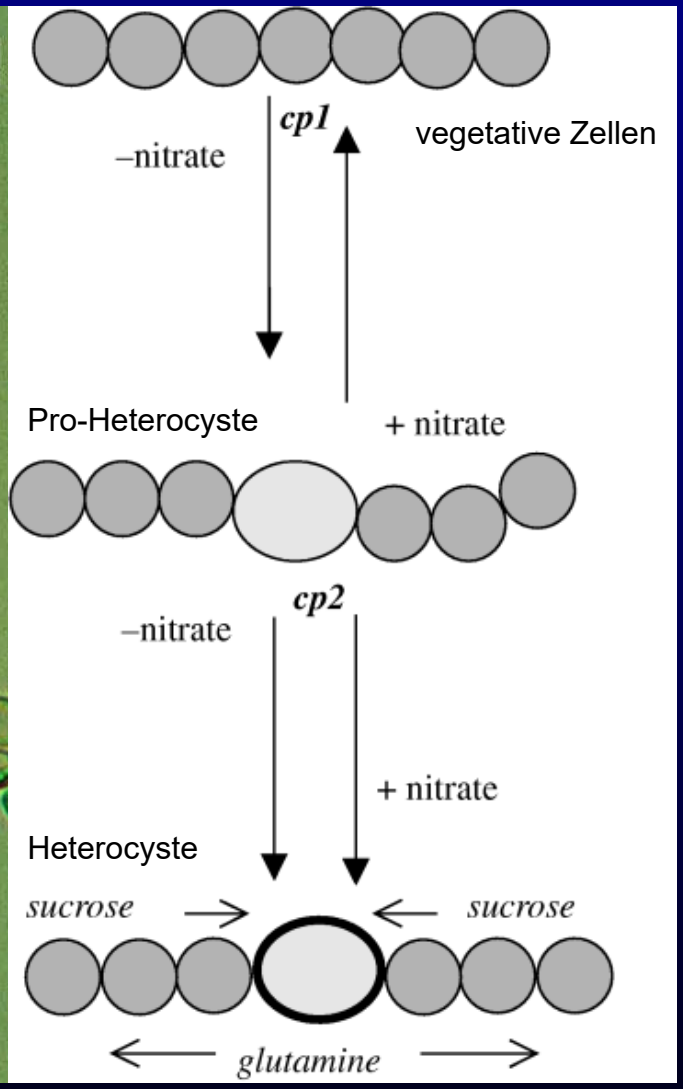
Regulation of photosynthesis for nitrogen fixation in unicellular cyanobacteria (II)



Heterocyst forming cyanobacteria



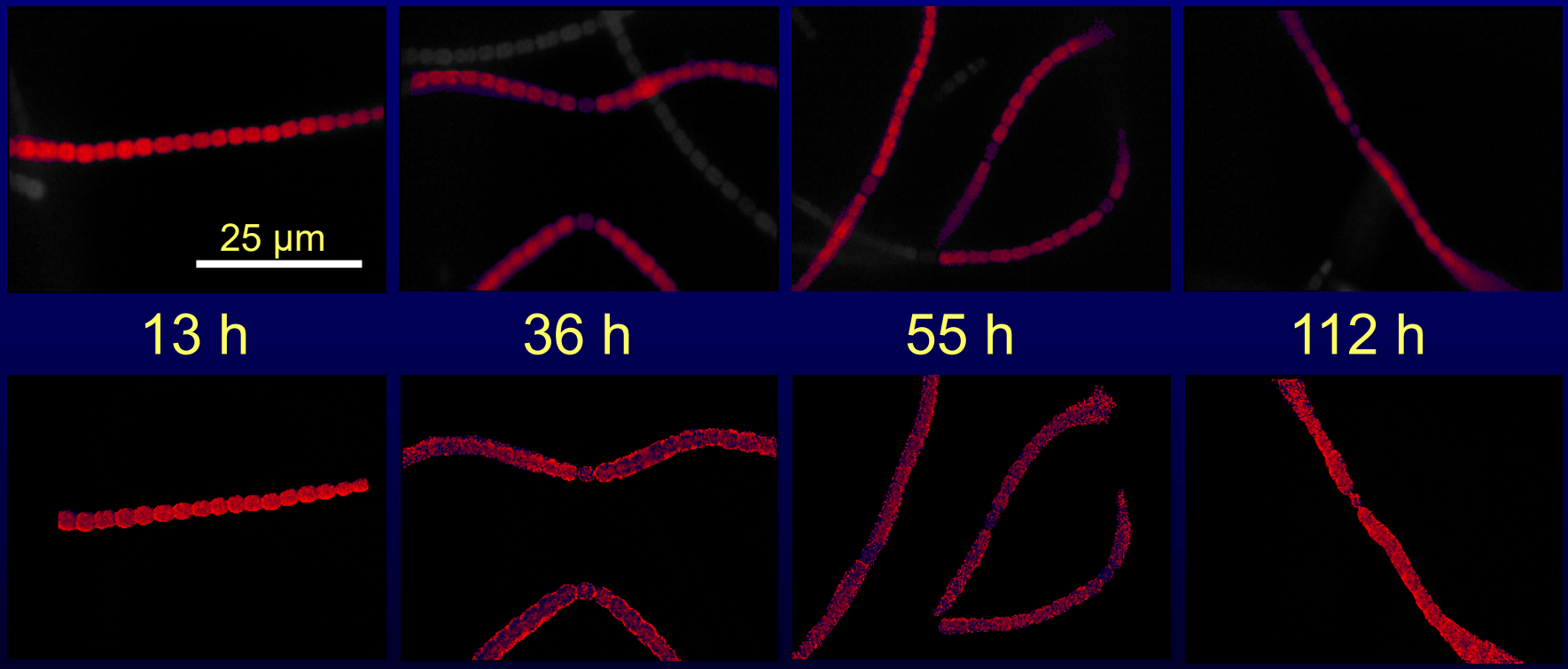
From: Culture service of Instituto de Bioquímica Vegetal y Fotosíntesis, Sevilla, Spain



From:El-Shehawy et al 2003 Physiol Plant 119 (1), 49-55

Heterocyst differentiation: distribution maps of chlorophyll fluorescence kinetic parameters

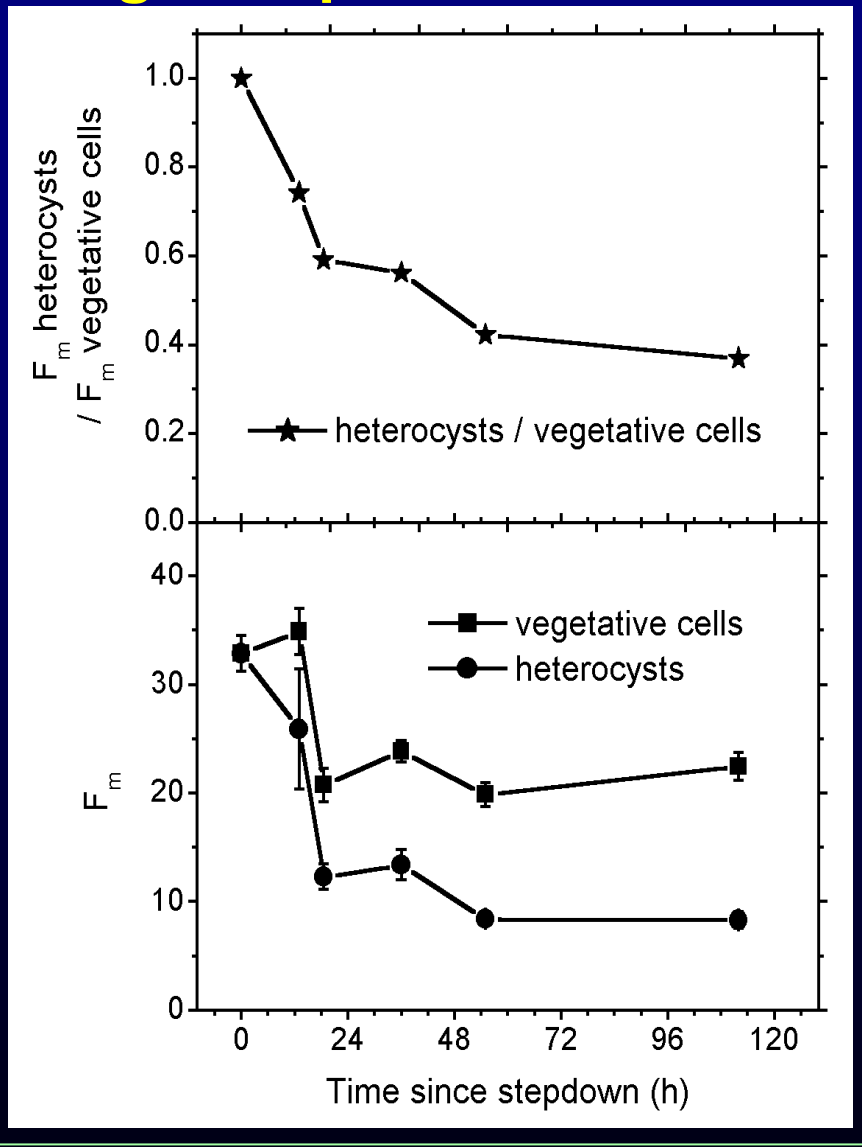
Maximal fluorescence quantum yield (F_m)

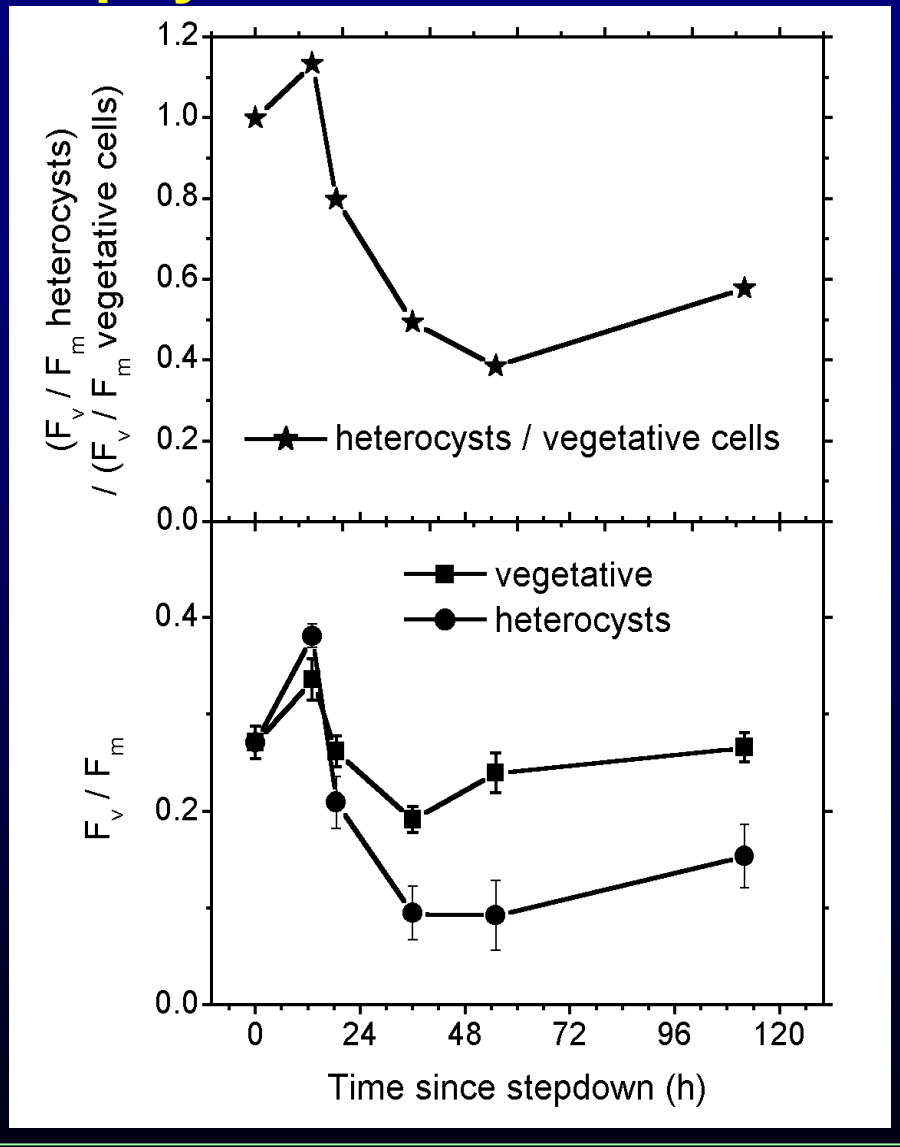


Photosystem II activity (F_v/F_m)

Ferimazova N, Felcmanová K, Šetlíková E, Küpper H, Maldener I, Hauska G, Šedivá B, Prášil O (2013) Photosynthesis Research 116:79–91

Heterocyst differentiation: changes in parameters of chlorophyll fluorescence kinetics





Ferimazova N, Felcmanová K, Šetlíková E, Küpper H, Maldener I, Hauska G, Šedivá B, Prášil O (2013) Photosynthesis Research 116:79–91

Trichodesmium:
anoxygenic photosynthesis energizing nitrogen fixation in the same cells during the photoperiod

Trichodesmium

- Marine filamentous, non-heterocystous cyanobacteria
- contribution of *Trichodesmium* to marine N_2 fixation: 30-50%
- Nitrogen fixation is confined to the photoperiod and occurs simultaneously with oxygenic photosynthesis.
- How nitrogenase is protected from damage by photosynthetically produced O_2 has remained an enigma.

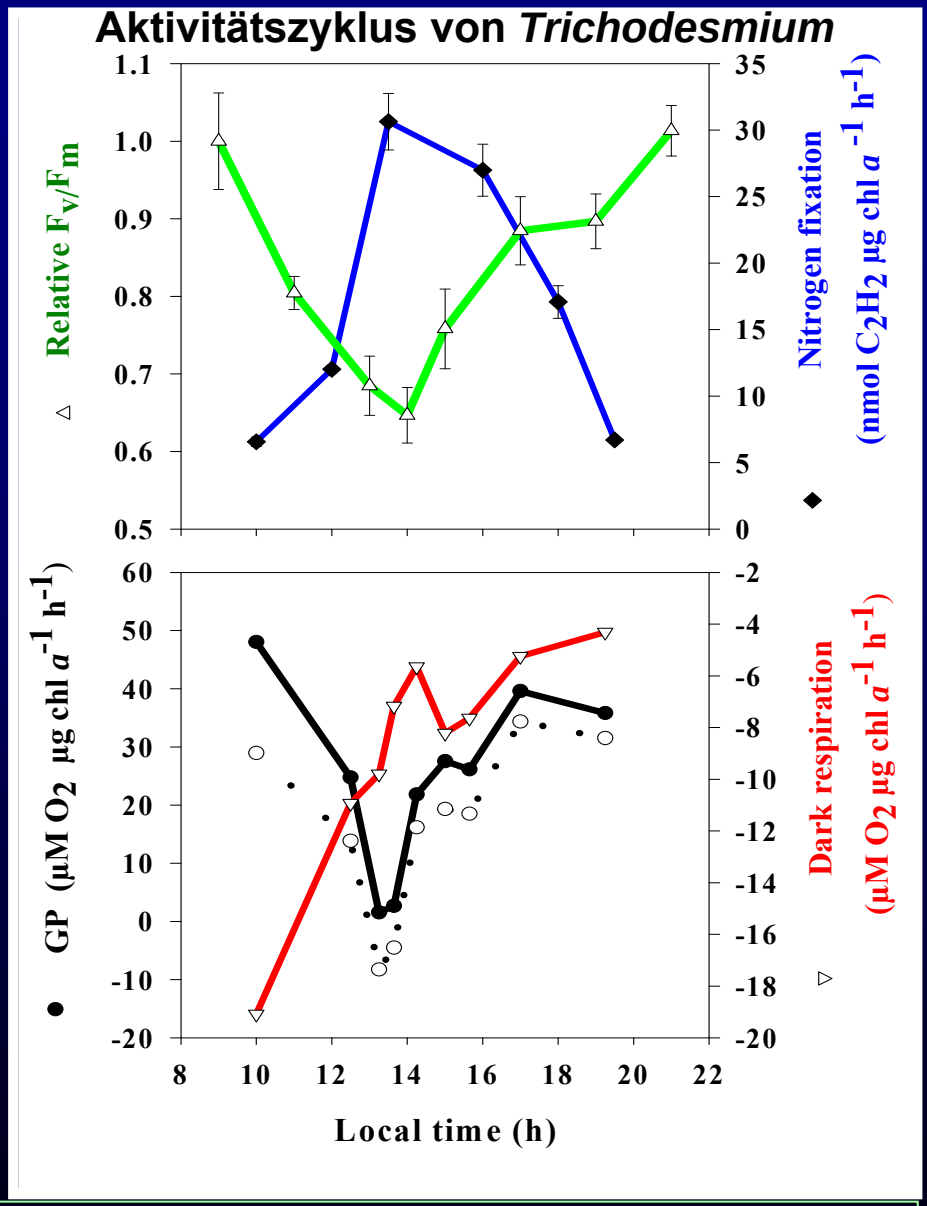
Surface blooms in the Arafura Sea

Colonies – tuft and puff formation

Trichodesmium

Trichodesmium-bloom

colonies: “Tuft” and “Puff”



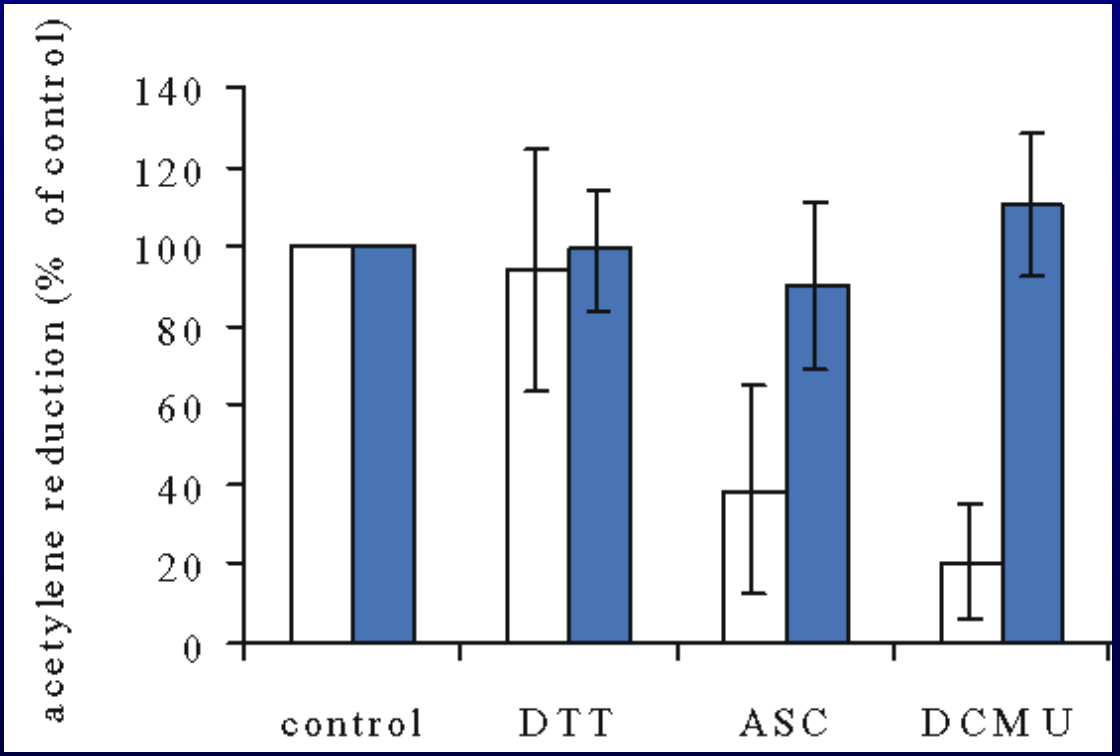
Berman-Frank I, Lundgren P, Chen Yi-B, Küpper H, Kolber Z, Bergman B, Falkowski P (2001) Science 294, 1534-1537

Co-Localisation of nitrogenase and PSII in *Trichodesmium*

D1 protein (green) and Nitrogenase (rot).
Big picture: Overlay, small picture: only nitrogenase (Immunostain)

Berman-Frank I, Lundgren P, Chen Yi-B, Küpper H, Kolber Z, Bergman B, Falkowski P (2001) Science 294, 1534-1537

Inhibitor-Tests: Need of PSII-activity for nitrogen fixation in *Trichodesmium*



Influence of DCMU (10 μM), ascorbic acid (100 μM), and DTT (100 μM) were tested for cultures incubated under aerobic (white columns) and anaerobic (blue columns) conditions. Changes in nitrogenase activity as measured by acetylene reduction.

Berman-Frank I, Lundgren P, Chen Yi-B, Küpper H, Kolber Z, Bergman B, Falkowski P (2001) Science 294, 1534-1537

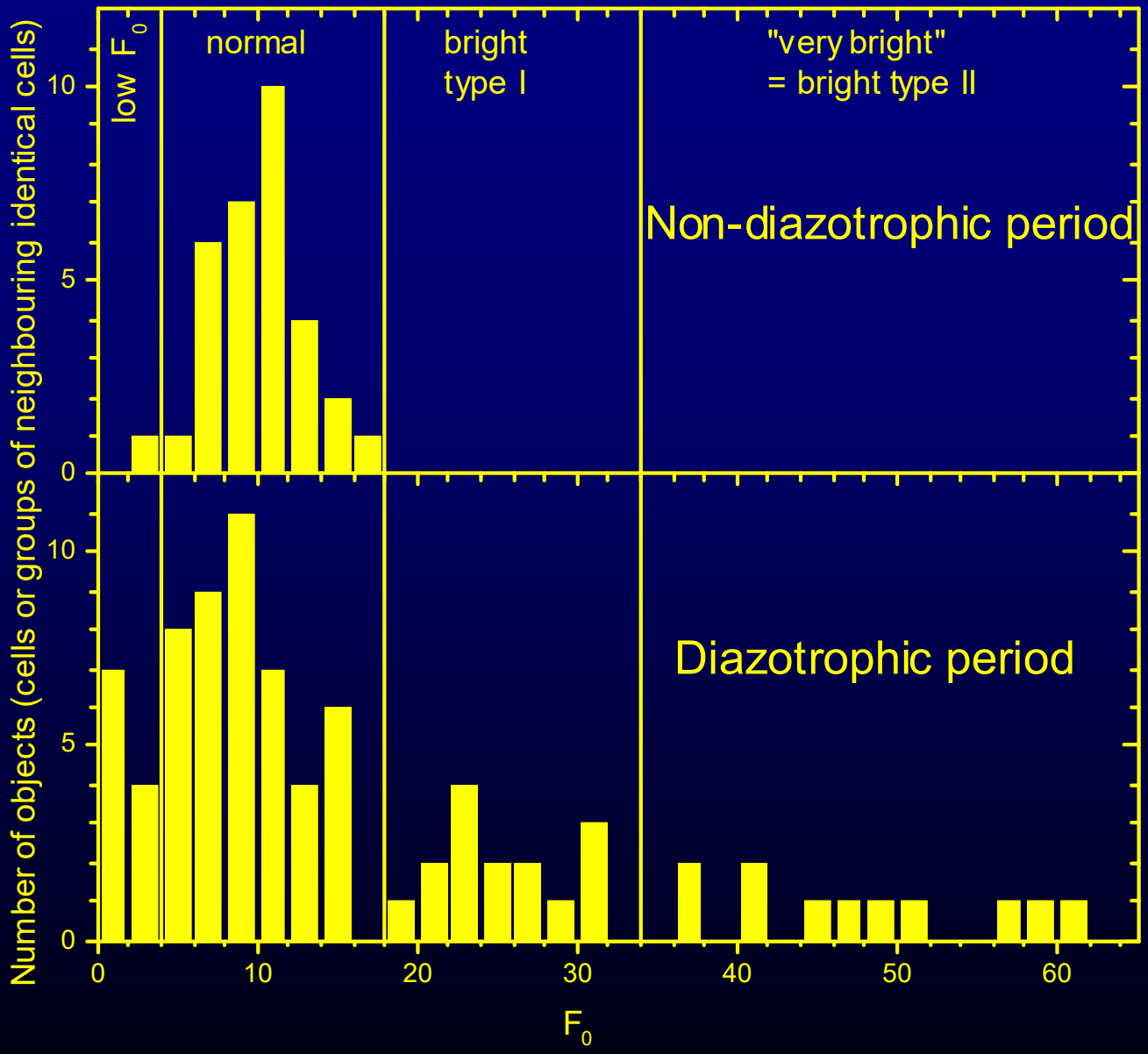
Proof of Mehler-reaction during nitrogen fixation

Staining with DAB (Diaminobenzochinon) shows intracellular distribution of H_2O_2 as brown stain in all cells

Mehler-reaction: $H_2O + 2O_2 \rightarrow H_2O_2 + O_2$

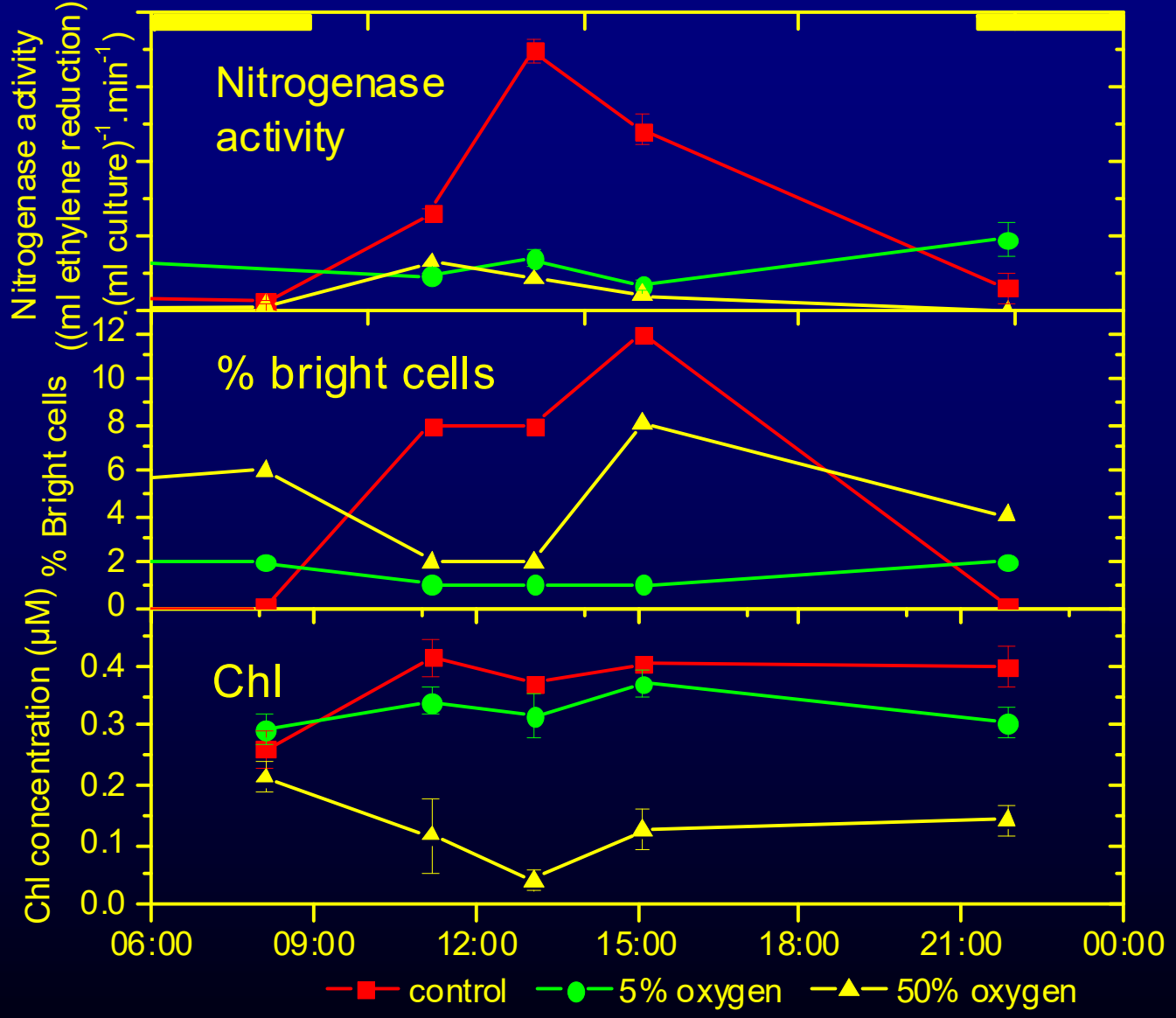
Berman-Frank I, Lundgren P, Chen Yi-B, Küpper H, Kolber Z, Bergman B, Falkowski P (2001) Science 294, 1534-1537

Diurnal cycle of activity: Distribution of F_0 values



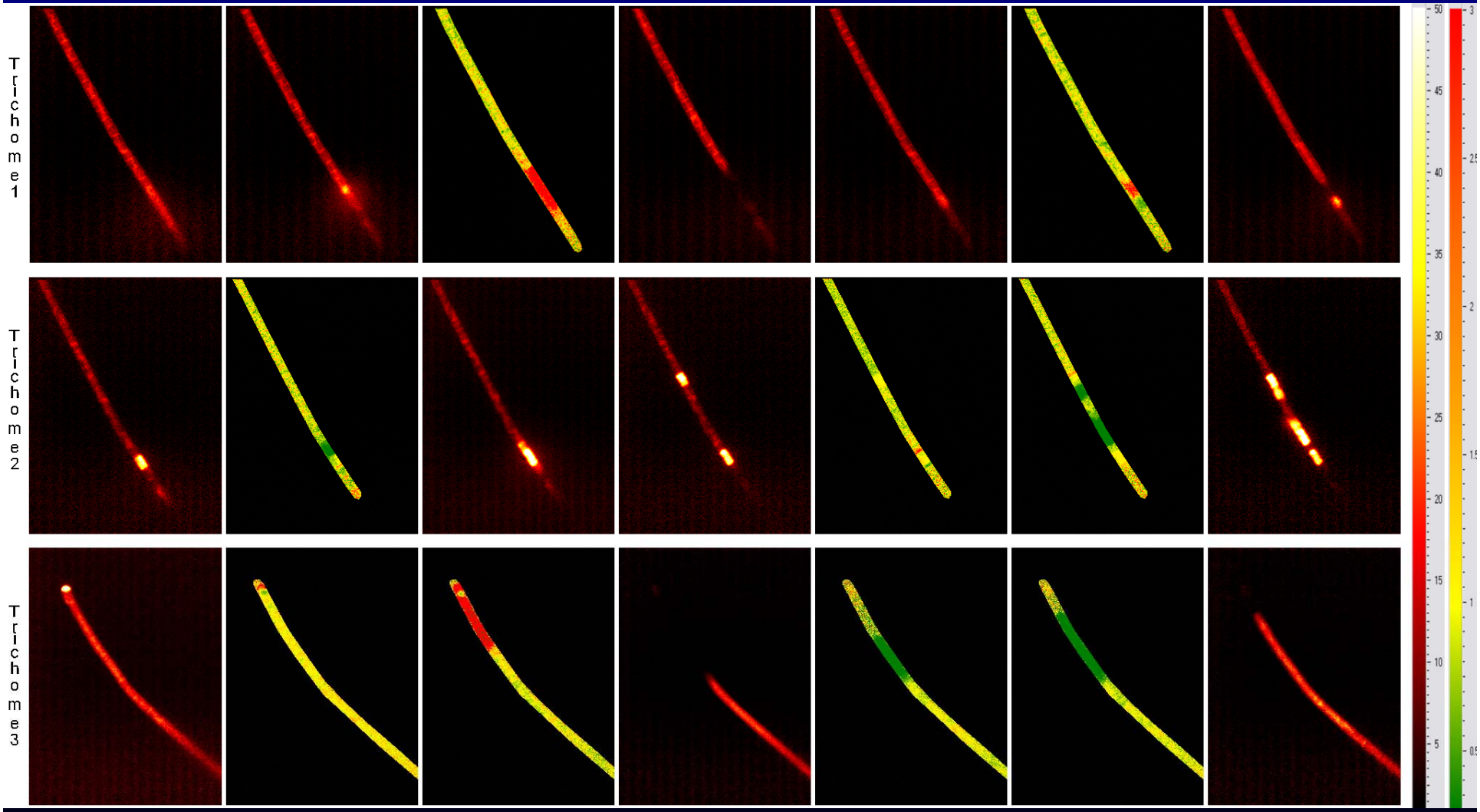
Küpper H, Ferimazova F, Setlík I, Berman-Frank I (2004) Plant Physiology 135, 2120-33

Diurnal cycle of activity: correlation between bright cells, pigment content and nitrogenase activity

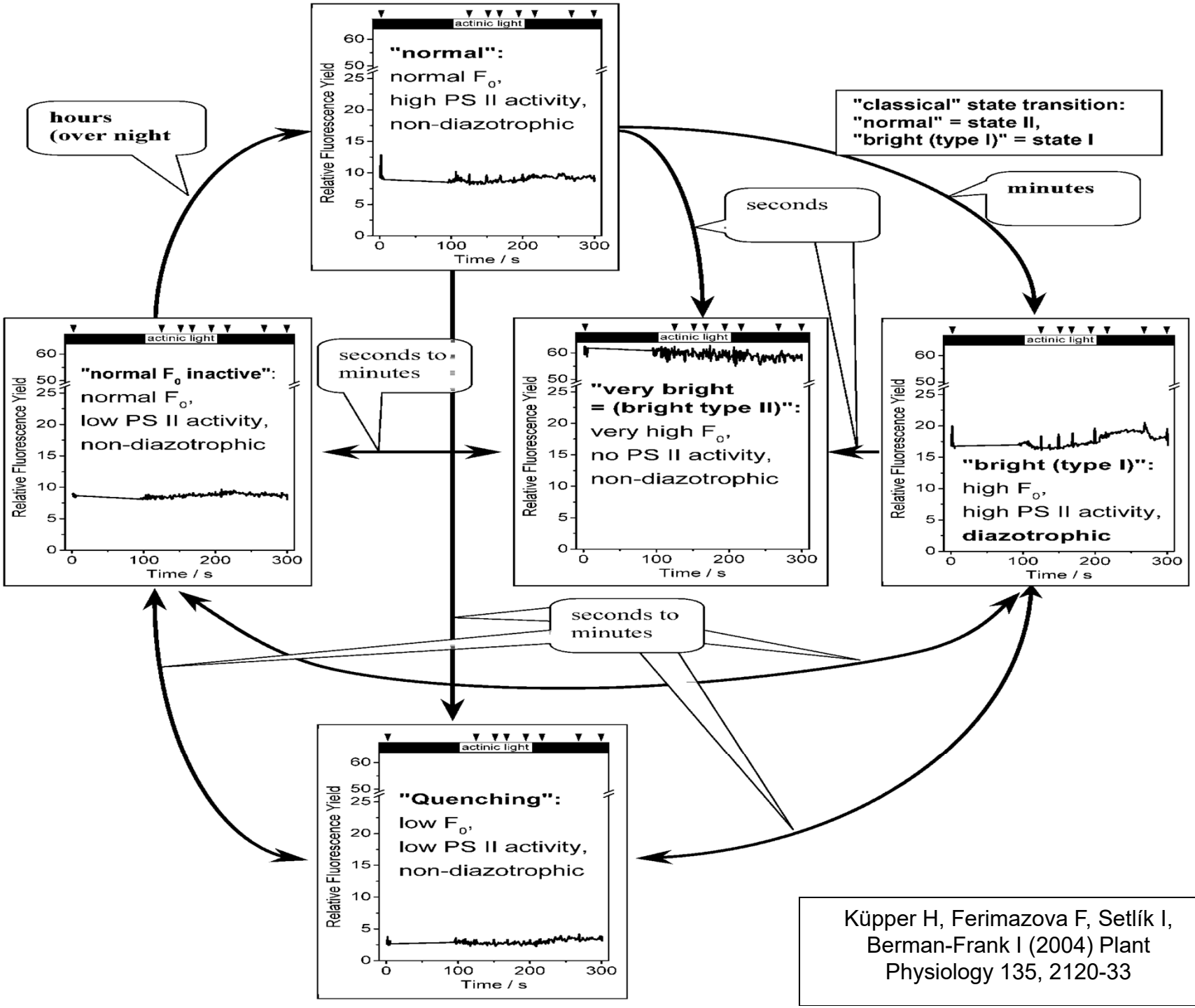


Küpper H, Ferimazova F, Setlík I, Berman-Frank I (2004) Plant Physiology 135, 2120-33

PSII-activity *Trichodesmium:* *reversibility of changes in fluorescence yield*



Küpper H, Ferimazova F, Setlík I, Berman-Frank I (2004) Plant Physiology 135, 2120-33



Küpper H, Ferimazova F, Setlík I, Berman-Frank I (2004) Plant Physiology 135, 2120-33

Hypothesis about the regulation of photosynthesis for nitrogen fixation in *Trichodesmium*

Light initiates photosynthesis

Reduction of the PQ pool

↓

Induction of a state 2 --> state 1-transition – cells have a high F_0 (“bright cells type I”) due to surplus phycobiliproteins

↓

Stimulation of pseudocyclic electron transport

↓

Energy and reduction equivalent for CO_2-assimilation

↓

High respiration rates in early light period

↓

Oxygen consumption exceeds oxygen production: Opportunity for nitrogen fixation

↓

Carbohydrates are consumed

↓

Respiration decreases, transition to the “normal F_0 inactive” or “quenching” state

↓

Intracellular oxygen rises

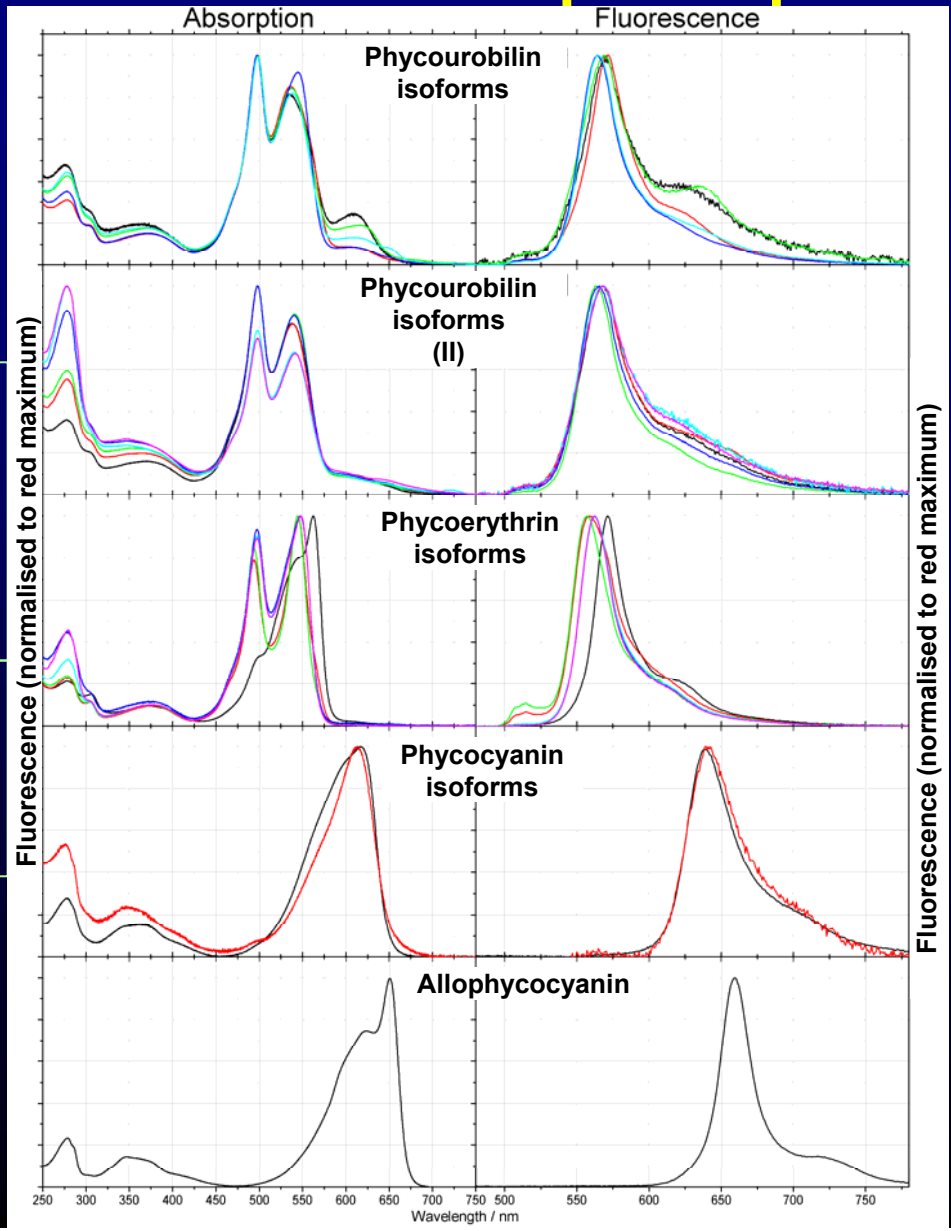
↓

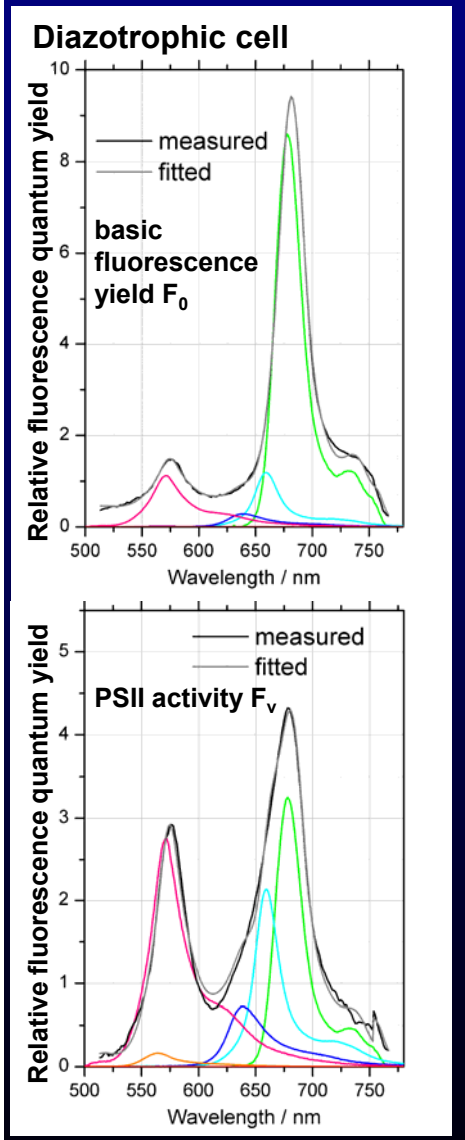
Nitrogenase becomes inhibited, no further nitrogen fixation until the following day, cells return to "normal F_0 active" state

Purification of *Trichodesmium* phycobiliproteins for deconvoluting spectrally resolved *in vivo* fluorescence kinetics and absorption spectra

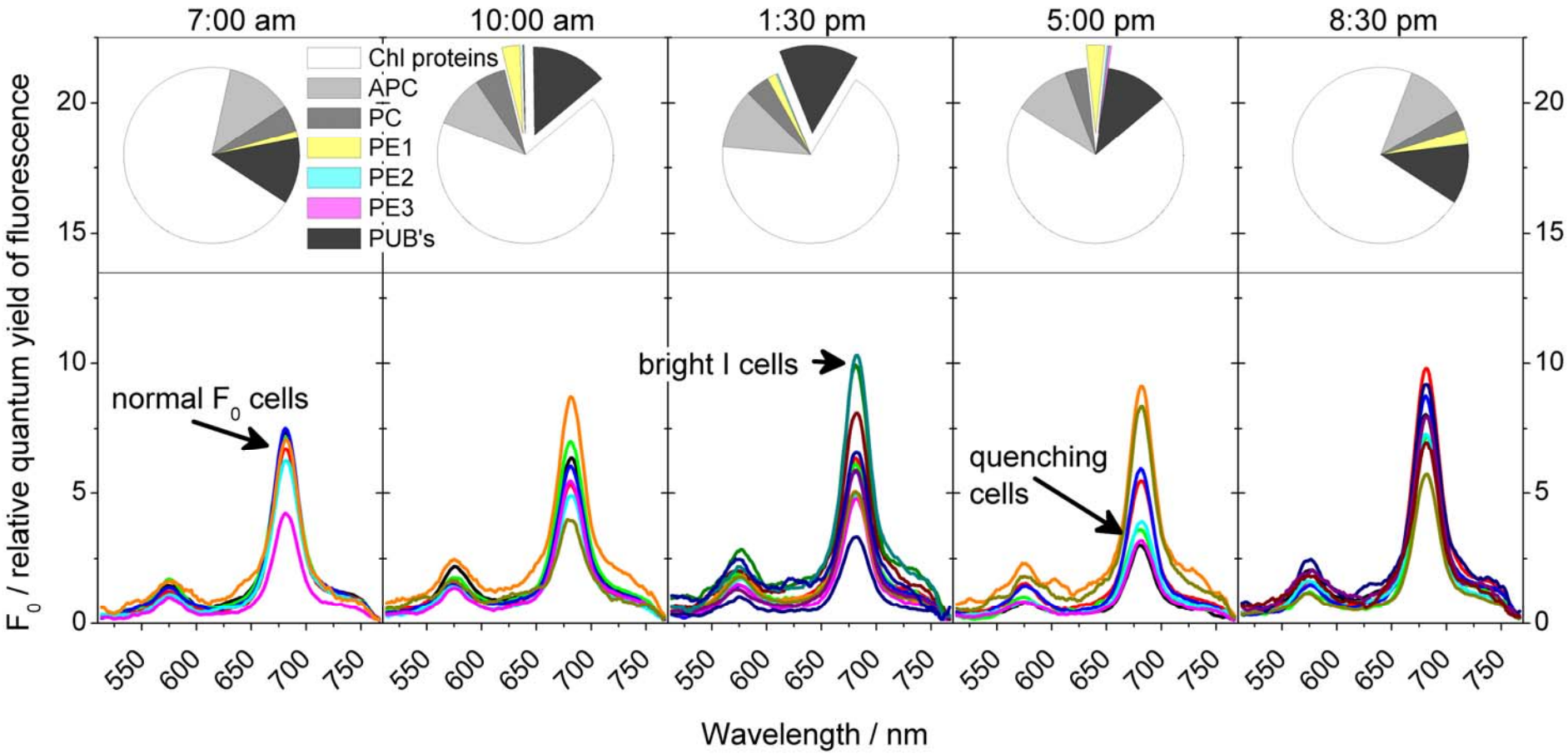
Phycobiliprotein purification + characterisation: Küpper H, Andresen E, Wiegert S, Šimek M, Leitenmaier B, Šetlík I (2009) Biochim. Biophys. Acta (Bioenergetics) 1787, 155-167

Method of deconvolution: Küpper H, Seibert S, Aravind P (2007) Analytical Chemistry 79, 7611-7627





Deconvolution of spectrally resolved *in vivo* fluorescence kinetics shows reversible coupling of individual phycobiliproteins



Küpper H, Andresen E, Wiegert S, Šimek M, Leitenmaier B, Šetlík I (2009) Biochim. Biophys. Acta (Bioenergetics) 1787, 155-167

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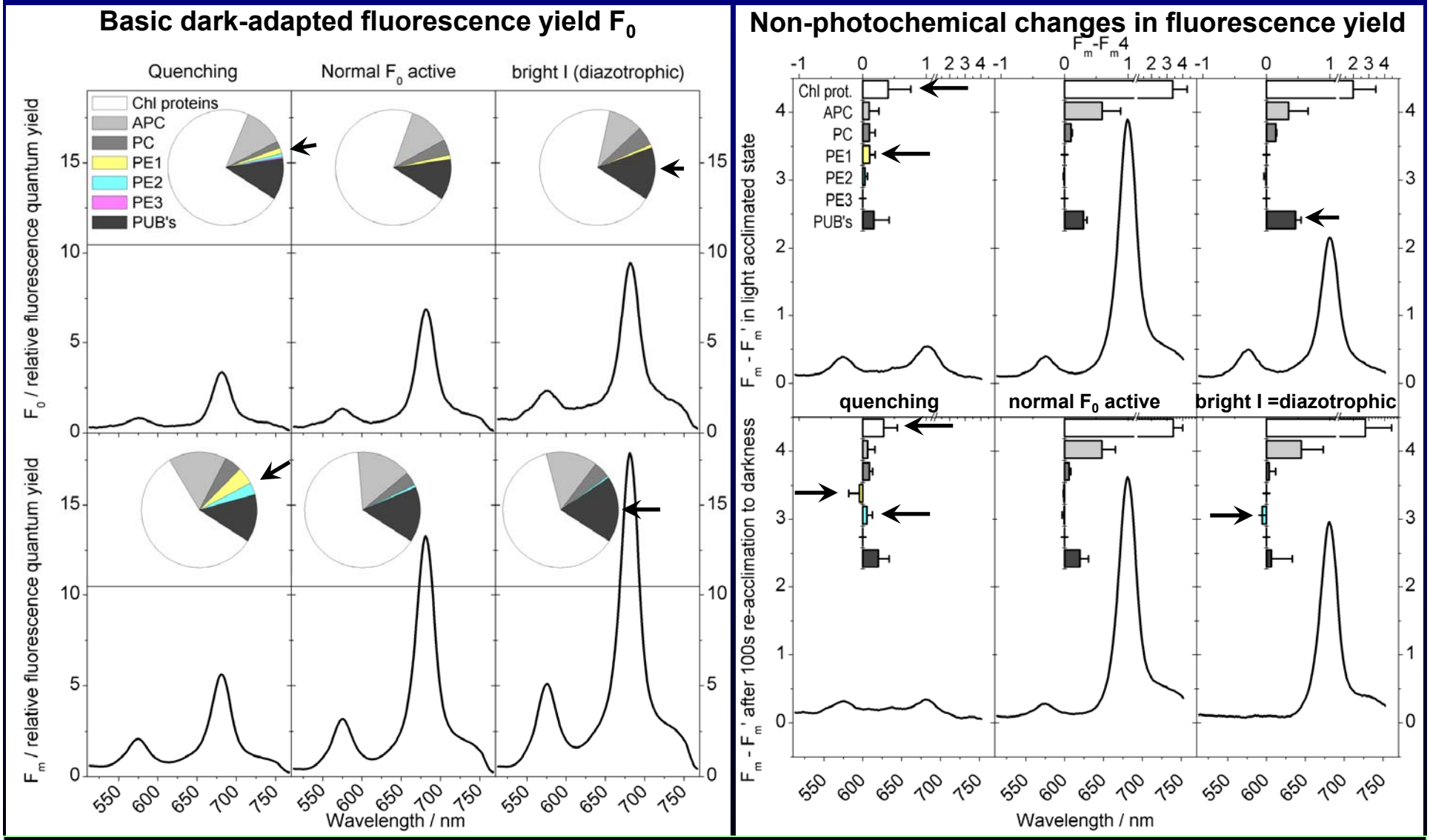
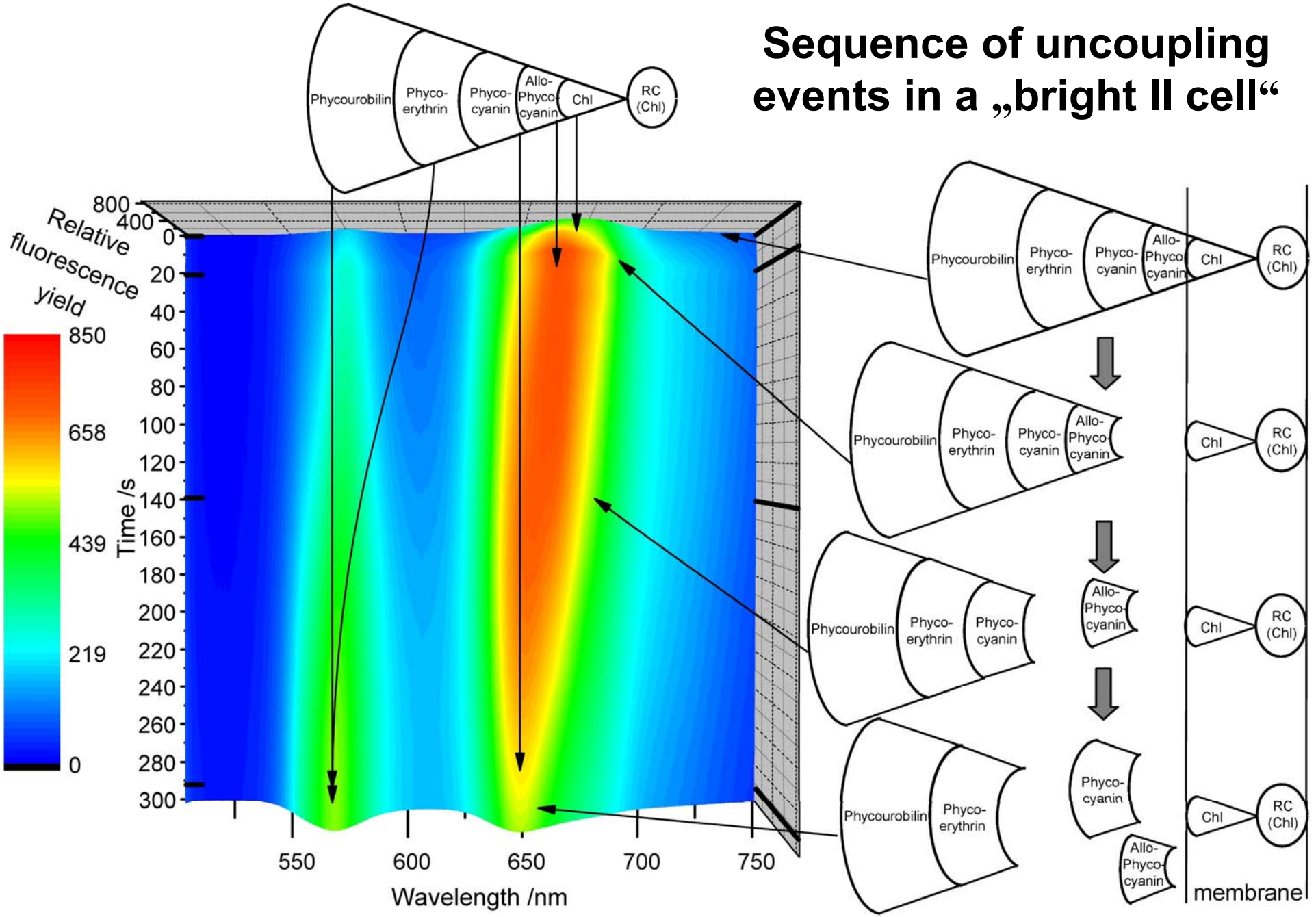


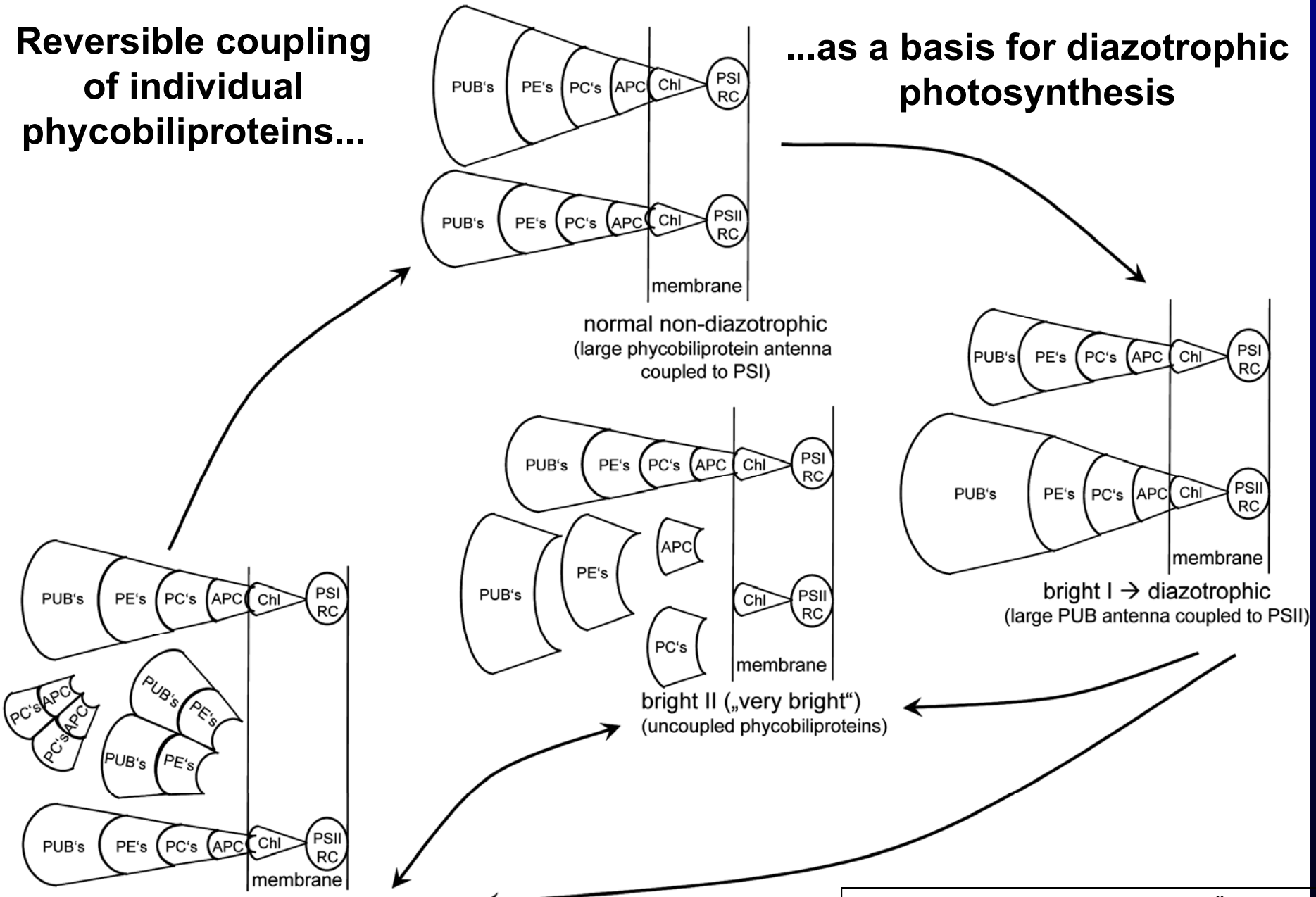
Küpper H, Andresen E, Wiegert S, Šimek M, Leitenmaier B, Šetlík I (2009) Biochim. Biophys. Acta (Bioenergetics) 1787, 155-167

Sequence of uncoupling events in a „bright II cell“



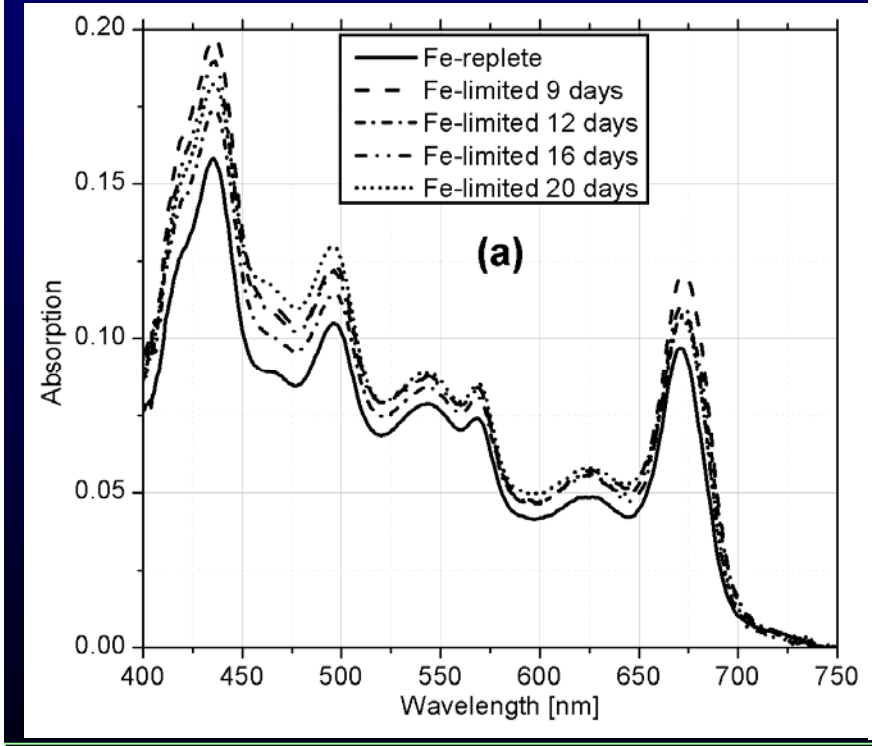
Reversible coupling of individual phycobiliproteins...

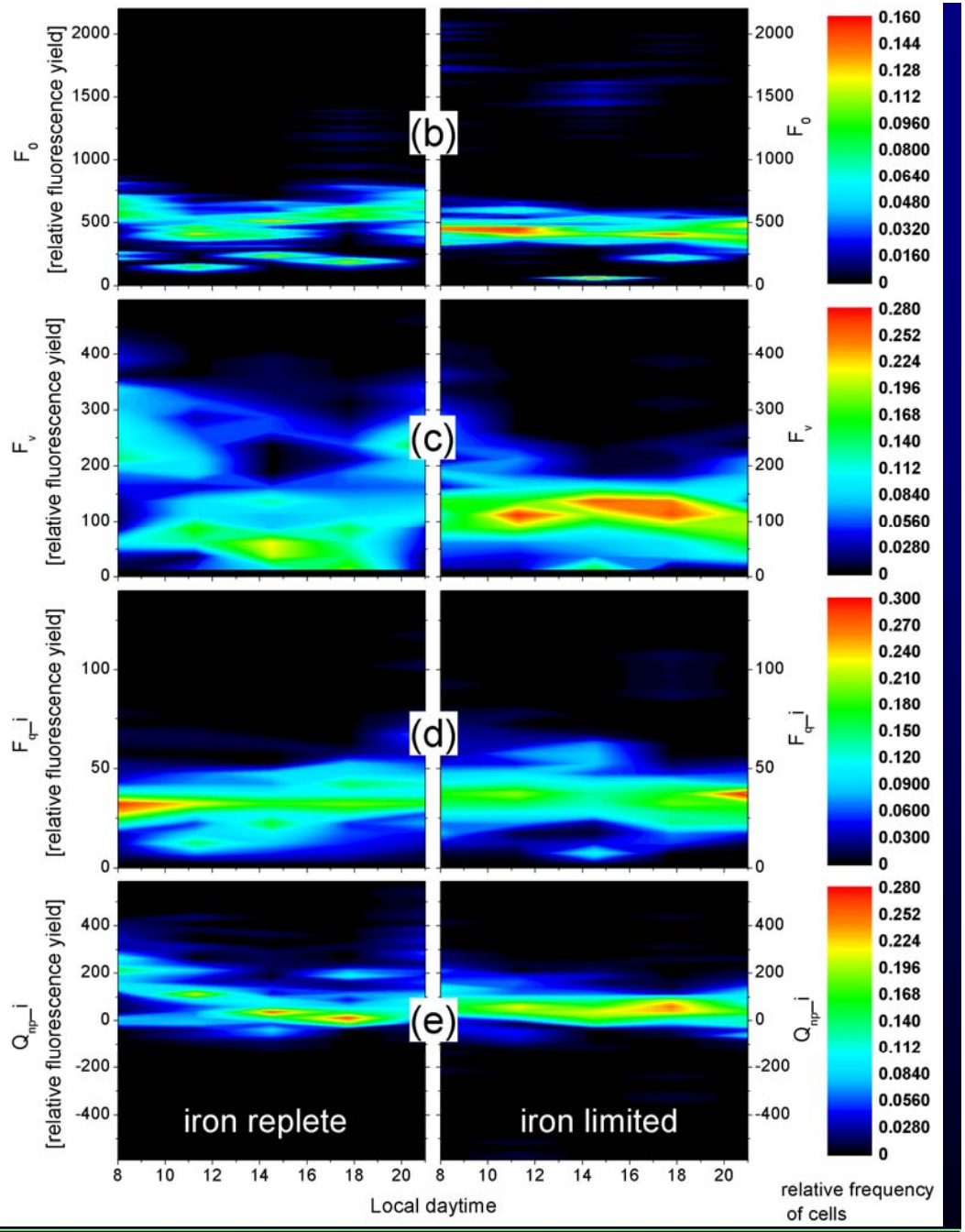
...as a basis for diazotrophic photosynthesis



Küpper H, Andresen E, Wiegert S, Šimek M, Leitenmaier B, Šetlík I (2009) Biochim. Biophys. Acta (Bioenergetics) 1787, 155-167

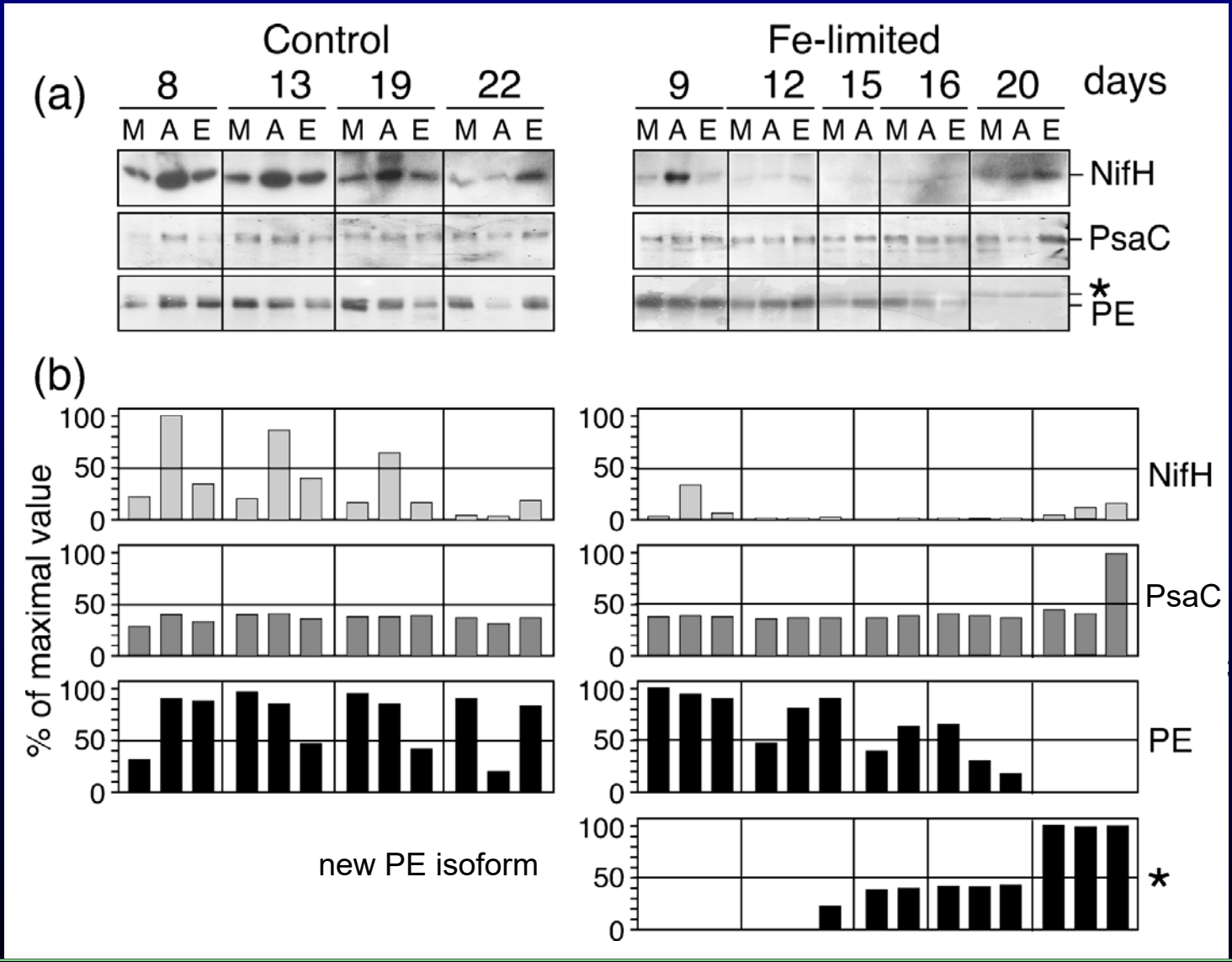
Iron limitation: photosynthetic components remain active...





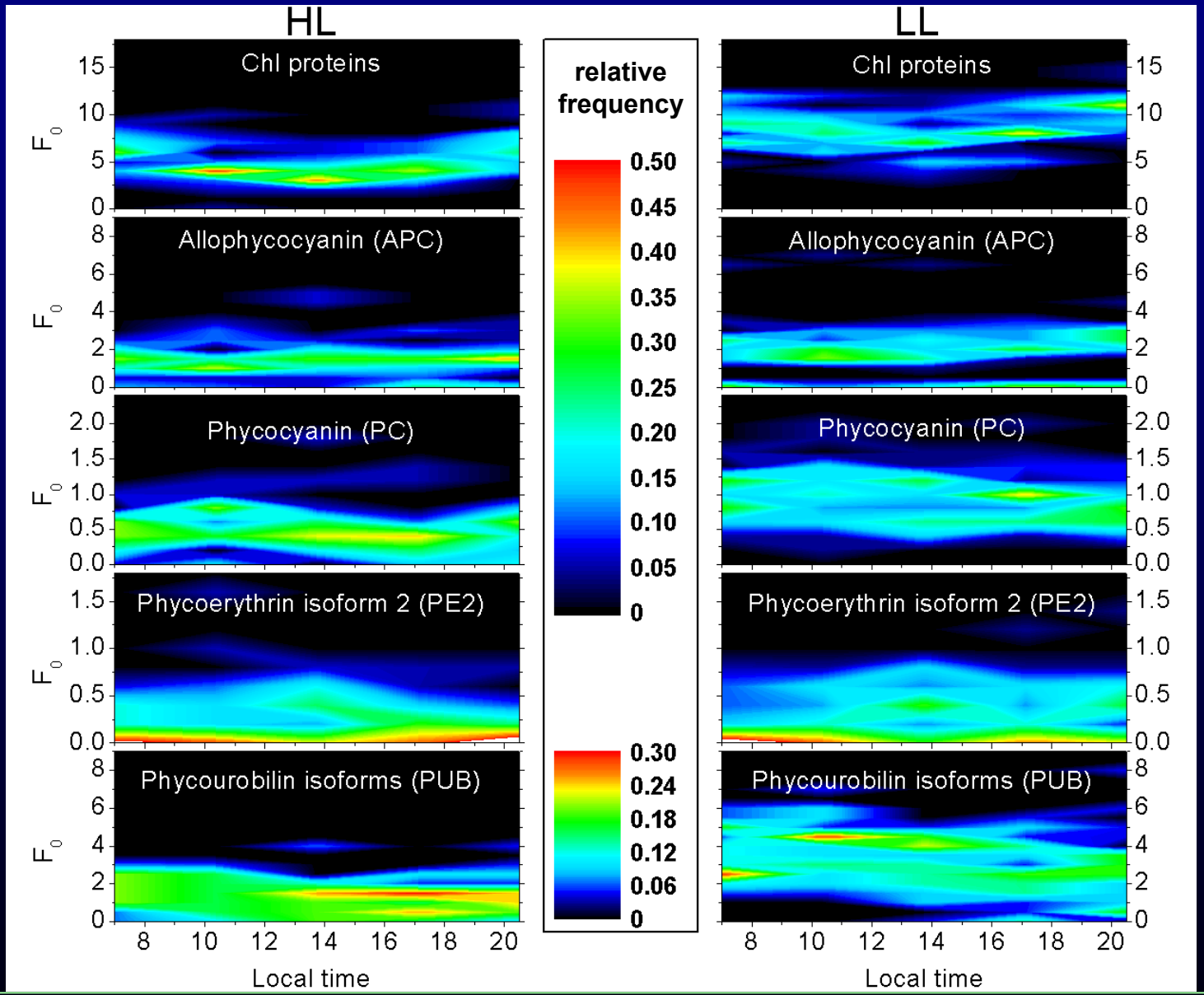
Küpper H, Šetlík I, Seibert S, Prášil O, Šetlikova E, Strittmatter M, Levitan O, Lohscheider J, Adamska I, Berman-Frank I (2008) New Phytologist 179, 784-798

Iron limitation: rescue of photosynthetic components...
...by sacrificing nitrogenase



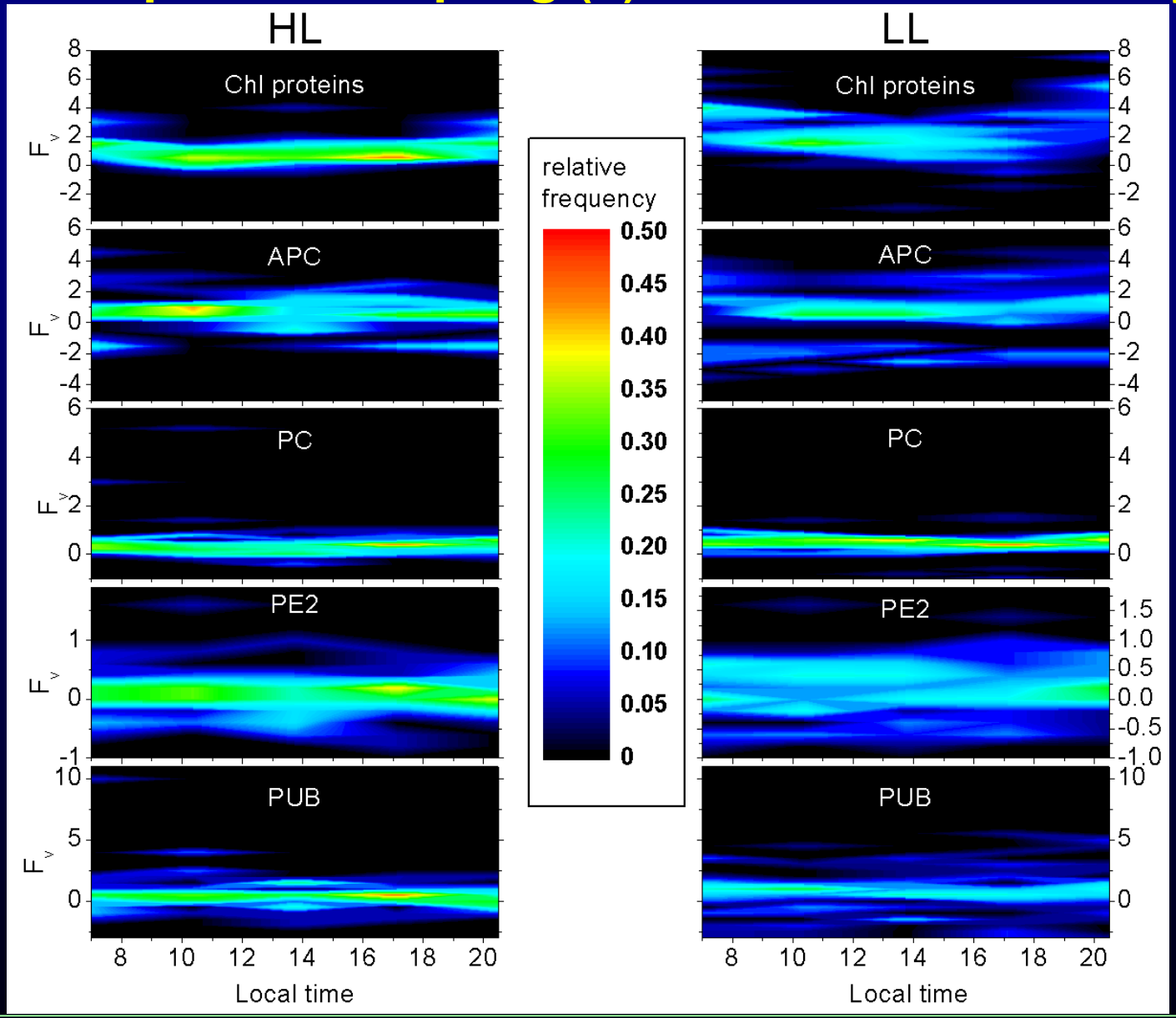
Küpper H, Šetlík I, Seibert S, Prášil O, Šetlikova E, Strittmatter M, Levitan O, Lohscheider J, Adamska I, Berman-Frank I (2008) New Phytologist 179, 784-798

Light limitation: acclimation by reversible phycobiliprotein coupling: basic fluorescence yield F_0



Andresen E, Adamska I, Šetlikova E, Lohscheider J, Šimek M, Küpper H, (2010) New Phytologist 185, 173-188

Light limitation: acclimation by reversible phycobiliprotein coupling (II): maximal PSII activity F_v



Andresen E, Adamska I, Šetlikova E, Lohscheider J, Šimek M, Küpper H, (2010) New Phytologist 185, 173-188

Conclusions

- For *Trichodesmium*, photosystem II activity is essential for providing energy for nitrogen fixation.
- Regulation of PSII activity for nitrogen fixation is achieved mainly by quickly reversible (un)coupling of individual phycobiliproteins.
- The nitrogen fixing activity state is characterised by a particularly large PSII-associated antenna, which is achieved mainly by coupling of additional units of phycourobilin (PUB) isoforms.
- Therefore, acclimation to light limitation ("low light stress") involves enhanced synthesis mainly of phycourobilin, which is then mainly coupled to PSII. Synthesis and levels of other photosynthetic components decrease.
- Because of their vital importance, when adverse conditions require a choice, *Trichodesmium* in contrast to other cyanobacteria does not sacrifice its phycobilisomes, but rather its nitrogenase.
- Stress leads to expression of alternative phycobiliprotein isoforms

All slides of my lectures can be downloaded from my workgroup homepage

Biology Centre CAS → Institute of Plant Molecular Biology → Departments → Department of Plant Biophysics and Biochemistry,

or directly

http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html